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Award Number: DAMD17-00-1-0713

TITLE: Dangerous Pathogens 2000

PRINCIPAL INVESTIGATOR: Les Baillie, Ph.D.

CONTRACTING ORGANIZATION: Defence Evaluation and Research Agency
Farnbourgh, Hants

REPORT DATE: December 2000

TYPE OF REPORT: Final Proceedings

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

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20010403 037

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	December 2000	Final Proceedings (1 Jul 00 - 31 Dec 00)

4. TITLE AND SUBTITLE
Dangerous Pathogens 2000

5. FUNDING NUMBERS
DAMD17-00-1-0713

6. AUTHOR(S)
Les Baillie, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Defence Evaluation and Research Agency
Farnbourgh, Hants

E-Mail:

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

14. SUBJECT TERMS
Conference

15. NUMBER OF PAGES
90

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

The rather grand sounding aim of the conference is encapsulated in the following paragraph; "At the beginning of the new millennium infectious disease is still a major scourge of the human race. The aim of this conference is to review the ongoing battle between man and microorganisms, past, present and future and to identify any gaps in our knowledge that need to be filled to ensure that man comes out on top".

The conference will be divided into the following themes;

The Past:

- Plagues of Antiquity
- Disease and War
- The Role of Sanitation and Clean Water
- The impact of Science

The Present:

- Plagues of the present
- Antibiotic resistant organisms
- Hospital acquired infections
- Global Vaccine programmes

The Future:

- Emerging Pathogens
- Genomics and Bioinformatics
- New antimicrobial agents

This meeting has been organised by DERA Porton Down and the Society for Applied Microbiology. We acknowledge the support of the following organisations;

Biogene, UK
Roche Diagnostics, UK
The Centre for Applied Microbiological Research, Porton Down, UK
The Defence Evaluation Research Agency, Porton Down, UK
The US Army Medical Research Acquisition Activity.

Tuesday 5th September — The Past

Plagues of Antiquity

Session Chairman Richard Manchee, DERA Porton Down, UK	9.00-9.10
Magna Pestilencia - Black Breath, Black Rats, Black Death Paul Russell, DERA Porton Down, Salisbury, UK	9.10-9.40
From Flanders to Glanders Tim Brooks, DERA Porton Down, Salisbury UK.	9.40-10.30
Tea	10.30-11.00
The History of Influenza Chris Potter, Medical Microbiology, University of Sheffield Medical School, UK.	11.00-11.25
Vaba, Haiza, Kholera, Foklune or Cholera: The Disease Well Known During 8 Pandemics. Prof. Duncan Stewart-Tull, Division of Infection and Immunity, University of Glasgow, UK.	11.25-11.50
Typhus as a Re-emerging Disease. Abdu Azad, University of Maryland School of Medicine, USA.	11.50-12.15
The Great Pox that was Syphilis. Armine Sefton, The London Hospital, London, UK.	12.15-12.40
Lunch	12.40 -14.00

The Impact of Clean Water and Science

Session Chairman Phil Baker, NIH, Bethesda, USA	14.00-14.10
Keeping it Clean: The Sparkling History of Murky Water Rosalind Stanwell-Smith, PHLS Communicable Disease Surveillance Centre, London, UK.	14.10-14.35
Life and Work of Sir Alexander Fleming Chris Collins, The Ashes, Hadlow, UK.	14.35-15.00
Tea	15.00-15.30
Jenner and the elimination of Smallpox Peter Beverley, The Jenner Institute, Newbury, UK	15.30-16.00
The Works of Louis Pasteur Maxime Schwartz, Institut Pasteur, Paris, France	16.00-16.30

Wednesday 6th September The Present

Modern Plagues - Eukaryotes, Viruses and Gram Negatives

Session Chairman Duncan Stewart-Tull, University of Glasgow, UK.	8.50 - 9.00
Coccidioides immitis as a "Select Agent" of Bioterrorism. Dennis Dixon, Division of Microbiology and Infectious Disease, NIAID, NIH, Bethesda, US.	9.00- 9.25
Development of Pre-erythrocytic stage vaccines against Plasmodium falciparum Malaria W.H.H.Reece, Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, UK	9.25 - 9.50
Vaccines to Combat the Two Faces of Influenza J.S. Oxford, St Bartholomew's and the Royal London School of Medicine and Dentistry & Retroscreen Ltd. London. UK.	9.50-10.15
HIV-1: Origins and Prospects. William Blattner, Institute of Human Virology, University of Maryland, Baltimore, US	10.15-10.40

Tea	10.40-11.00
Eradication of Biological Polio David Wood, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts, UK.	11.00-11.25
Cholera Toxin; Friend or Foe Prof. Tim Hirst, Department of Pathology and Microbiology, University of Bristol, UK	11.25-11.50
Bubonic Plague in the world today Di Williamson, DERA Porton Down, Salisbury, UK	11.50- 12.15
Lunch	12.15-14.00

Modern plagues-Gram positives

Session Chairman Phil Hanna, University of Michigan, USA	14.00-14.10
Tuberculosis, Current Problems, Future Prospects Douglas B Young, Imperial College School of Medicine, London, UK.	14.10-14.35
Antibiotic Resistant Staphylococcus Aureus: Back to the Future? Georgia Duckworth, CDSC, London UK.	14.35-15.00
Enterococci: *Opportunists par excellence?* J. Wade, Dulwich Public Health laboratory & Medical Microbiology, Guy's King's & St Thomas' School of Medicine, King's College Hospital, London, UK.	15.00-15.25
Bacillus anthracis, a bug with attitude ! Les Baillie, DERA Porton Down, Salisbury, UK	15.25- 1550
Tea	15.50-1615
Poster session	16.15-17.00

Thursday 7th September — The Future

Emerging Pathogens

Session Chairman Les Baillie, DERA Porton Down, Salisbury, UK	8.50-9.00
New viral enemies: fact or fiction ? Graham Lloyd, CAMR Porton Down, Salisbury, UK	9.00-9.25
Evolution of a Bacterial Pathogen: Mobile Genetic Elements and the Emergence of Pathogenic V. Cholerae B.M. Davis, Howard Hughes Medical Institute and Division of Geographic Medicine and Infectious Diseases, New England Medical Center and Tufts University School of Medicine, Boston, US.	9.25-9.50

Genomics and Bioinfromatics

Session Chairman Jennie C Hunter-Cervera. University of Maryland Biotechnology Institute, Maryland US	9.50-10.00
Pathogen Sequencing: The Role of Malaria Genome Sequencing in Antimalarial Drug and Vaccine Development. Dan Carcucci, Director of Genomics and Bioinformatics Malaria Program, Naval Medical Research Institute, Bethesda, US.	10.00-10.15
Genome Sequencing of Yersinia Pestis Julian Parkhill, The Sanger Centre, Cambridge, UK.	10.15-10.30
The Surprising Cholera Sequence John Heidelberg, The Institute of Genomic Research, Rockville, US.	10.30-10.45
Tea	10.45-11.00
Analysis of the Partial genome Sequence of Francisella Tularensis Strain Schu 4 Rick Titball, Technical Manager, DERA Porton Down, UK	11.00-11.15
The Secrets of the Bacillus Anthracis Genome Revealed Timothy Read, The Institute for Genomic Research, Rockville, US	11.15-11.30
Bioinformatics: Information is power or too much knowledge is a dangerous thing Mark Pallen, Department of Microbiology and Immunology, The Queen's University of Belfast, Grosvenor Road, Belfast UK	11.30-11.55

Using proteomics to explore pathogen survival responses David O'Connor, Division of Biochemistry & Molecular Biology, School of Biological Sciences, University of Southampton, UK	11.55-12.20
Lunch	12.20-13.45

Therapeutics **Session chairman** Tim Read, The Institute for Genomic Research, Rockville, US	**1345-1355**
Microbial Genomics- A revolution in antimicrobial Drug-hunting A.E.Allsop, Astra Zeneca, Alderley House, Alderley Park, Macclesfield, Cheshire, UK	**13.55-14.20**
Are antiviral agents a realistic option in control of dangerous viruses ? cost and risk versus benefit C.R.Penn Centre for Applied Microbiology and Research (CAMR), Porton Down, Salisbury, UK	**14.20-14.45**
Emerging vaccines technologies: exciting prospects for new products S.N. Chatfield, Microscience Ltd, 545 Eskdale Road, Winnersh Triangle, Wokingham, Berkshire, UK	**14.45-15.10**
Tea	**15.10-15.30**
Preventing deliberate disease: future control regimes Graham S Pearson Department of Peace Studies, University of Bradford, West Yorkshire, UK.	**15.30-15.55**
Overview Harry Smith, University of Birmingham, UK	**15.55-16.20**

Tuesday 5th September 2000

The past

MAGNA PESTILENCIA- BLACK BREATH, BLACK RATS, BLACK DEATH

P. Russell, CBD Porton Down, Salisbury, Wiltshire SP4 0JQ

In the history of medicine, nothing has exemplified the devastation caused by infectious disease as the second plague pandemic. The outbreak captures the imagination of both medical and historical scholars alike and serves as a warning for the future should an untreatable, infectious disease spread among a vulnerable, ignorant population.

The lack of contemporary records and diagnostics means that the pandemic cannot be proven to have been plague. The limited information available, however, does describe the clinical pictures of bubonic, septicaemic and pneumonic plague. The causative organism, *Yersinia pestis*, was carried by fleas themselves carried on rats and also apparently on swatches of cloth. It is widely accepted that the disease arose in central Asia during the mid 14^{th} century and spread throughout the Eurasian landmass causing huge depopulation and profound social and economic change. The scale of the disease led to persecution and self-mutilation for religious appeasement but also stimulated the foundations of public health and medical research unhindered by religious prohibitions.

The reasons for the sudden appearance of the disease, its spread and its subsequent disappearance are subject to numerous theories and conjecture. Applying modern ideas of disease emergence to the historical events and situations of the time reveal that many factors were 'in place' which would facilitate the rapid spread of virulent disease. The biological characteristics of Y. pestis, its flea vector and its rodent reservoir makes plague a prime candidate as the disease which took advantage of these conditions.

FROM FLANDERS TO GLANDERS

Lt Col Tim Brooks, RAMC, Consultant Microbiologist, CBD Porton Down.

Infectious disease has been a key factor in military operations since the earliest times, and until the late 20th century accounted for more casualties than any form of enemy action. Endemic disease has always taken a heavy toll on soldiers, and as empires expanded an increasing number of individuals found themselves away from their native lands and exposed to new disease to which they had little natural resistance. Thousands of Europeans lost their lives to malaria and yellow fever in Africa and the New World, and the problems of disease spurred on research into the transmission, prevention and the treatment of these diseases. Thus, the British Army played a key role in identifying the life cycle of malaria, and the first anti-malarial drugs were introduced in Allied troops in the Borneo campaign in 1943. Following the work of Ross and Manson on the transmission of malaria, British forces developed a variety of procedures for disease control including vector control. The threat of malaria has remained until the present, when over 70 members of the detachment sent to Sierra Leone in 2000 developed falciparum malaria.

Epidemic disease remains the oldest friend of war and warriors, and apart from military losses it can devastate a population. The conditions of war with poor housing, sanitation and food supplies encourage endemic disease to become epidemic, and new epidemics may arrive with refugees or troops. Plague, cholera and smallpox have all made a significant impact on history in this way. Epidemic typhus was a major problem in the trenches in World war I and appeared again in concentration camps in WWII. Unusual diseases can also be fostered by the conditions of war, so that Trench foot was a major cause of morbidity in the First World War and again in the Falklands campaign.

War also gives opportunities to exploit disease to a nation's advantage. The deliberate spread of smallpox and plague are historical examples, and more recent attempts include the biological sabotage campaign waged by the Germans in Europe and the US, in which glanders was deployed as a weapon against horses. The Japanese experimented widely with Biological Warfare in WW2, and the UK, US and Canada had active campaigns in the 1940s and 50s. In recent times a number of nations have development a biological weapons capability, and there are threats of terrorists following suit. Infectious disease therefore remains as a key feature of warfare for the foreseeable future.

THE HISTORY OF INFLUENZA

CW Potter, Medical Microbiology, Division of Molecular and Genetic Medicine, University of Sheffield Medical School, Beech Hill Rd. Sheffield S10 2RX.

Because of the well recognised characteristics of epidemic influenza, the history of this infection can be traced back reliably for three hundred years, probably for five hundred years and anecdotally throughout history. The past presents a repeating pattern; and since nothing has occurred to affect this pattern, it remains a template for the future. Influenza is an epidemic infection occurring in most countries in some years and in some countries in most years. The infection is severe with a mortality rate of one per thousand. This rate may appear low, but given that twenty or more percent of the population may be infected in an epidemic, deaths numbering 1-10 or more thousand in year occur commonly in the United Kingdom.

Epidemics are the result of antigenic changes in the virus: the virus accumulates mutations such that protective antibody acquired as a result of previous infection but does not protect, or only partially protects, against subsequent infection. More dramatically, every ten to forty years since AD 1700, recognisable pandemics have occurred which spread world-wide from a single point to involve the populations of most countries in a period of months: infection rates are very high with mortality figures of a million or more. The virus strains causing these pandemics are unrelated to preceeding virus types, and do not arise from them by mutation: these viruses arise by the reassortment of two influenza viruses, a human strain and an avian strain, multiplying in a third species. In the past century, pandemics occurred in the AD1900, 1917, 1957 and 1968; and the pattern over the past three hundred years suggests that a new pandemic will occur within the next decade. Descriptions of the epidemics of the twentieth century indicate our expectations of the future.

VABA, HAIZA, KHOLERA, FOKLUNE OR CHOLERA: THE DISEASE WELL KNOWN DURING 8 PANDEMICS

Prof. Duncan Stewart-Tull, Division of Infection & Immunity, Institute of Biomedical & Life Sciences, Joseph Black Building, University of Glasgow, Glasgow, G12 8QQ, Scotland.

The origins of cholera are variously listed from 400BC to 700AD (Macnamara, 1876) but there is general agreement that it "emerged" as a new disease for western civilisations in May 1817, With the outbreak in India, where the disease is endemic. This was the start of the first pandemic which spread rapidly and few countries avoided the high mortalities. In 1875, Peters reported that infected women unable to move formed a dam for hundreds of dead bodies during the monsoons. Birds and wild dogs gorged on the flesh from the dead lying in the fields.

Some horrific remedies were proposed: drinking cow's urine three times a day, throwing pepper in the eyes and the application of hot irons to the soles of the feet among many, (Baron, 1958). In the UK some physicians recommended injecting saline solutions or drinking large volumes of water. Many more people were to die, however, in the seven pandemics before oral rehydration therapy (ORT) was rediscovered in the middle of the twentieth century.

Today some believe that the eighth pandemic looms but more likely large outbreaks may be restricted to countries where peace, the provision of clean water and efficient sewage disposal schemes are still unattainable. With current air travel good vaccines and vaccination procedures are required to restrict the spread of disease from such outbreaks.

Peters, J.C. (1875) A history of the travels of Asiatic cholera in Asia and Europe.
Baron, A.L. (1958) Man against germs. Robert Hale Ltd., London.
Macnamara, C. (1876) A history of Asiatic cholera. London.

TYPHUS AS A RE-EMERGING DISEASE

Abdu F. Azad, Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD, USA 21201.

The genus Rickettsia includes bacterial agents responsible for some of the most historically significant and severe diseases of humans. These include Rickettsia prowazekii, the causative agent of epidemic agent typhus, R. typhi, responsible for murine or endemic typhus, R. rickettsii, the agent of Rocky Mountain spotted fever and several others, responsible for a variety of spotted fevers occurring world-wide. Rickettsial diseases, widely distributed throughout the world in endemic foci with sporadic and often seasonal outbreaks, from time to time have re-emerged in epidemic form in human populations. Throughout history, epidemics of louse-borne typhus have caused more deaths than all the wars combined. The ongoing outbreak of this disease in Burundi involving more than 30,000 human cases, in South America, and Russia is a reminder that rickettsial diseases can re-emerge in epidemic form as a result of the catastrophic breakdown of social conditions and environmental alterations. In addition to explosive epidemics, sporadic but limited outbreaks of other rickettsial diseases have been reported throughout the world. In the United States, a drastic increase of murine typhus in the 1940s, Rocky Mountain spotted fever (RMSF) in the late 1970s, and the human ehrlichioses in the 1990s attests to the potential emergence of these infections in populations at risk. The rickettsial obligate intracellular existence in both mammalian and anthropod hosts serves as an excellent model to study the complex host-parasite interactions. The ecological separation and reduced selective pressure provided very stable associations between rickettsiae and their invertebrate vectors. Thus, opportune conditions allow the rickettsial emergence and re-emergence in areas where the cycles thrive. Deciphering the interacting components of rickettsial cycle provide clues to predict rickettsial emergence and to control the transmission cycle thereby preventing epidemics. In this presentation, host/rickettsial cycle will be analysed from historical, ecological, and molecular prospective.

THE GREAT POX THAT WAS - SYPHILIS

Dr Armine Sefton, Department of Medical Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry, London E1 2AD, United Kingdom

The "Great Pox", syphilis, is a systemic disease caused by the spirochaete *Treponema Pallidum*. It is sometimes referred to as "The Great Mimic" since it can have so many clinical manifestations. It is usually transmitted sexually but congenital infections can occur. In addition, in certain parts of the world endemic non-venereal disease due to *Treponema pallidum* occurs. Controversy exists as to the historical origins of venereal syphilis. However, there are three main theories. The most common theory, often referred to as the "Columbian Theory" is that Columbus brought it back from the New World on his return to Europe in 1493. The second theory, the pre-Columbian theory, is based on the fact that European medical literature in the 1200-1300s describes certain forms of "leprosy" which were highly contagious and could be transmitted sexually and from mother to child in-utero. This form of "Leprosy" was said to respond to mercury and may have been syphilis. The least known theory, the Evolutionary Theory, postulates that the different members of the genus Treponema evolved from a single organism responding to changes in the environment. The first use of salvarsan in 1909 was a breakthrough in the therapy of syphilis.

KEEPING IT CLEAN: THE SPARKLING HISTORY OF MURKY WATER

R Stanwell-Smith, Communicable Disease Surveillance Centre, Public Health Laboratory Service, 61 Colindale Avenue, London, NW9 5EQ, United Kingdom.

Water related disease is as old as recorded history, and perhaps because of this long experience, lessons tend to be either taken for granted for forgotten. We can identify some 'watersheds' in the trends of controlling water borne pathogens and in providing accessible, safe domestic supplies. The contemporary influences on emerging water pathogens include climate change, different patterns of host susceptibility, increased population mobility, changing water behaviours and the various expectations and demands of water suppliers, from whirlpool spas to iced water on tap in the work place. In addition to our recent problems regarding the control of *Cryptosporidium spp.* In water supplies, global travel can bring the still prevalent traditional water pathogens uncomfortably close to home. So, what can we learn from history? We can note, first, that it took nearly two millennia to extend, to most of our cities, the brilliant work of Frontinus, the water commissioner who described the Roman aqueduct system in 97AD[1]. The New River company laid down pipes in 1619 in London[2], but it was not until 1900 that piped water became common in even the upper and middle class homes of Europe and America. The decades leading up to 1900 can now be seen to be significant watershed in three important areas for water related disease: the transition from the miasma to the germ theory; the combined impact of public health and the application of epidemiology; and the emergence of reliable water and sewage treatment and management. One outbreak encapsulates this shift: the water borne epidemic of typhoid fever in Maidstone, Kent, 1897[3]. It was the first in which chlorination was used to control a suspected contamination supply, but also the first example of a trial of typhoid vaccine and there are other messages still relevant to out concern with modern supplies and emerging water threats. This outbreak will be presented from the perspective of 'murky water' stories before and after this incident.

References

1.Herschel, Clemens. The two books on the water supply of the City of Rome of Sextus Julius Frontinus. 1899, Boston: Dana Estes.
2.Turneaure F E, Russell H L. Public water supplies. 1924, New York: John Wiley.
3Borough of Maidstone. Epidemic of typhoid fever, 1897. Report of an inquiry and appendices. London, HMSO, 1898.

LIFE AND WORK OF SIR ALEXANDER FLEMING

C.H. Collins. The Ashes, Hadlow, Kent, United Kingdom.

Fleming became a medical student almost by accident, chose his school of medicine on the basis of sport, qualified as surgeon but never practised, became a bacteriologist not quite accidentally and "discovered" penicillin only because a fungal spore accidentally entered his laboratory through a window. All very happy accidents for posterity!

Much has been written about Fleming and his biography is, rightly, lengthy one, so this short review is based on a personal interest in the career of a man whom I met only once as an examinee, but who left a lasting impression.

JENNER AND THE ELIMINATION OF SMALLPOX

P.Beverley The Edward Jenner Institute for Vaccine Research, Compton, Newbury, UK

Abstract not submitted

THE WORKS OF LOUIS PASTEUR

M. Schwartz, Institut Pasteur, Unité de Physiologie Cellulaire, Département des Biotechnologies, Paris, France.

It is my great privilege to convey to you, tributes, thanks and respect from all involved in medicine and surgery; it is true to say that, of all the people in the world to day, medical sciences owe you the most.... For centuries, infectious diseases have been shrouded, as it were under a dark curtain. In discovering the microbial origin of disease you have raised that dark curtain!

These were the words of Joseph Lister, addressing Louis Pasteur on the occasion of his 70^{th} birthday, during a ceremony held at the Sorbonne, in Paris, on Dec. 27. 1892.

Louis Pasteur was indeed the creator, together with Robert Koch, of the microbial theory of infectious diseases. However, he did much more. He was the founder of stereochemistry, he demonstrated the role of microorganisms in fermentation and putrefaction, and established the basis for the manufacture of vaccines. The consequences of his work are far reaching. They can still be felt today, in science, in industry, in agriculture, in veterinary and human medicine, and in many other aspects of our life.

Wednesday 6th September 2000

The present

COCCIDIOIDES IMMITIS AS A "SELECT AGENT" OF BIOTERRORISM

Dennis M. Dixon. Ph.D., National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 20892. US.

Coccidioides immitis is the most virulent of the primary fungal pathogens of humans and other animals. As such, it is the only fungal etiological agent designated as Biosafety Level 3 in *Biosafety in Microbiological and Biomedical Laboratories*, 3rd Ed. (HHS Publication Number [CDC] 93-8395; http://www.nih.gov/od/ors/dspubs/bmbl/index.htm). Natural infection occurs via inhalation of aerosolised conidia from a primary environmental focus, and can also occur from traumatic implantation. In both instances, analogies can be drawn to *Bacillus anthracis*. Similarly, *C. immitis* was listed amongst the " select agents" in the Final rule on Additional Requirements for Facilities Transferring or Receiving Select Agents in response to the U.S. Antiterrorism and Effective Death Penalty Act of 1996 (42 CFR Part 72; Federal Register, Vol. 61 No. 207, 1996). Natural infection can resolve spontaneously following a primary pulmonary, flu-like episode, or can progress to a serious and life-threatening local or disseminated disease. Natural or experimental infection appears to confer immunity, and efforts are underway to develop a protective vaccine. Such would be a welcomed addition to the limited antifungal armamentarium.

DEVELOPMENT OF PRE-ERYTHROCYTIC STAGE VACCINES AGAINST *PLASMODIUM FALCIPARUM* MALARIA

W.H.H.Reece,P.Gothard,V.Moorthy,K.Watkins,N.Arulanantham.M.T.M.Roberts, J.Schneider ,C.Hannan, S.C.Gilbert, M.Plebanski and A.V.S. Hill Nuffield Department of Medicine, John Radcliffe Hospital, Oxford, OX3 9DU,UK

Several attempts have been made to construct recombinant vaccines against *Plasmodium falciparum* malaria that mimic the protective immunity in humans induced by vaccination with irradiated sporozoites. Such protective vaccines would most likely induce IFNgamma secreting T-cells specific for pre-erythrocytic stage epitopes from *P.falciparum* (Schneider *et al.,* 1999).

We therefore used the IFNgamma ELISPOT and cytotoxicity assays to screen several candidate vaccines for immunogenicity in mice, and also examined protective efficacy in the murine *P.berghei* model of human *P.falciparum* infection. Several delivery systems, routes of administration, regimen and antigens were assessed, and in most cases, immunogenicity was linked to protection (Plebanski *et al.,* 1998; Gilbert *et al.,* 1999). A two vaccine regime of priming with DNA followed by boosting with recombinant MVA (Modified Vaccinia Ankara strain) was chosen to test in humans (Schneider *et al.,* 1998). The human constructs contained an ME (Multi-Epitope) string of epitopes to which naturally exposed African people have already shown to have specific cytotoxic T-cells. In addition, two promiscuous T helper cell epitopes (one from Tetanus Toxoid, and one from BCG) were included in the construct (Gilbert *et al.,* 1997). This ME-string was linked to whole TRAP (Thrombospondin Related Anonymous Protein) antigen from *P.falciparum* strain T9/96.

A Phase 1 open-label safety and immunogenicity study of these constructs was conducted in which DNA by either intramuscular injection or Powderject's needleless delivery device, and MVA injected intradermally. All the vaccines were safe with no serious adverse events observed.

T cell immunogenicity results by ELISPOT indicate that both DNA and MVA induce IFNgamma secreting cells specific for epitopes from various regions of the vaccined construct. Most of the responses were found to peptides from TRAP, and to the two helper epitopes in the ME string. The sizes of the responses were extremely variable between individuals, as might be expected from an outbred human population. The protective efficacy of the vaccines is currently being studied, based on a heterologous challenge of vaccinated volunteers with the 3D7 strain of *P.falciparum* and results from this study will be available for presentation.

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Plebanski,M. *et al.,* (1998).*European Journal of Immunology* 28:4345-4355
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Schneider,J. *et al.,* (1999). *Immunological Reviews* 170:29-38

VACCINES TO COMBAT THE TWO FACES OF INFLUENZA

J.S. Oxford, St. Bartholomew's and the Royal London School of Medicine and Dentistry & Retroscreen Ltd. London. UK.

Influenza A uniquely of any human virus has two epidemiological faces, the yearly epidemic and the infrequent pandemic. Influenza vaccines have contributed to preventing disease and infection in both scenarios since 1946. No vaccines were available in 1918 for the great Spanish Influenza Pandemic.

Current inactivated influenza vaccines prevent hospitalisation and death in the elderly and the 'at risk'. Important new antiviral drugs have been developed but vaccines will remain the cornerstone of prevention of this important disease.

Influenza pandemics may smoulder for 6-12 months before exploding. This lead-time gave opportunities in 1957 and 1968 to manufacture vaccine. The advent of new cell culture systems should speed up the production of pandemic vaccines. However, advance planning is needed to identify groups in the community to be given the first doses. Should these groups include the elderly or should preference be given to younger member upon whom a community is very reliant? Who will administer vaccine and where? Will rich advanced countries of the world buy up vaccine for their own citizens, thus depriving other communities entirely?

There are important scientific advances to be made in the yearly epidemic influenza vaccines. Could the HA and NA subunit be delivered directly to the respiratory mucosa? Are there subgroups of 'non-responders' in the community and could the immune system of these groups be manipulated to enhance T or B cell responses. Are there correlations with HLA types?

Influenza vaccines have been a scientific success story for 50 years. The extensive practical experience with administration of vaccine campaigns on a year to year basis will also help in pandemic planning.

HIV-1: ORIGINS AND PROSPECTS

William A. Blattner, MD. Institute of Human Virology, University of Maryland, Baltimore, MD, USA

Based on phylogenetic analyses, the Human Immunodeficiency Virus (HIV). A lente retrovirus, is thought to have entered man through enzootic transmission from a primate source some time in the 1930's. By the time that the current pandemic was recognized in 1981, a large pool of infections have been established in many parts of the world. HIV-1 is almost universally fatal with untreated patients progressing to terminal immunodeficiency on average with in 10 years in developed countries and perhaps half the time in developing world. Since the beginning of the epidemic over 53 millions persons have become HIV positive, almost 19 million have died and more than 34 million are alive with HIV-1 infection today world wide. In countries with access to care, combination antiviral therapy has led to declines in morbidity and mortality, but multi-drug resistance threatens this progress. Effective, sustainable, and affordable therapies are urgently needed for the 90% of patients world wide with out access to therapy. Antiretroviral therapy, given to women and their infants at birth substantially reduces HIV transmission. However new infections among adults continue at a rate of approximately 10 new infections per minute in 1999 despite extensive prevention efforts. The goal of a safe and effective preventative vaccine has been elusive because of the above noted malleability of the virus, and the lack of known correlates of protective immune response.

First generation vaccines are in clinical trials, and a number of innovative strategies employing novel vaccine delivery approaches and development of immunogens with possible cross-neutralization potential. However, achieving the goal of developing and widely disseminating an effective vaccine under the most optimistic of scenarios is at least 7 to 10 years in the future.

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ERADICATION OF BIOLOGICAL POLIO

David Wood, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts, EN6 3QG, UK.

The eradication of wild-type polioviruses is biologically plausible since there are no animal reservoirs, the viruses survive for relatively short times in the environment, excretion is generally for a short period only and there are two highly effective vaccines available. A global polio eradication initiative launched in 1988 has almost turned this theoretical possibility into reality. By the end of the year 2000 the World Health Organisation expects that more than 190 countries and territories will have interrupted transmission of wild polioviruses. This represents more than a 95% decline in incidence since the start of the polio eradication initiative in 1988 (1). Intensified eradication efforts are being made in the 30 countries that remain endemic to ensure that the goal of global polio eradication is achieved. The strategies that have been used are (a) high (>80%) routine coverage with oral poliovaccine (OPV), (b) national immunisation days to flood communities with OPV, (c) use of standardised surveillance indicators to monitor progress, and (d) targeted mopping-up OPV campaigns to eliminate the last remaining foci transmission. After eradication is achieved, re-introduction wild polioviruses into a community from laboratory stocks would represent a public health disaster of global importance. For this reason the WHO has developed a global action plan for containment of polio laboratory stocks (2). This calls for progressively higher levels of laboratory containment and envisages that wild poliovirus will be treated as a biohazard level 4 organisms in the not too distant future. Laboratories are encouraged to destroy unwanted wild polio stocks and to transfer important isolates to designated repositories. Finally, an additional benefit of polio eradication is that immunisation against polio may stop. How and when this should occur is not yet clear (3) and further research is necessary to build consensus on what will probably be one of the most difficult decisions of the entire process. The current status of the eradication initiative, laboratory containment plans, and research on stopping polio immunisation will be reviewed.

(1) RB Alyward et al, (2000), Bull WHO, 78, 285-297
(2) WHO (2000), in press
(3) DJ Wood et al, (2000), Bull WHO, 78, 347-363

CHOLERA TOXIN: FRIEND OR FOE?

T.R. Hirst, Department of Pathology & Microbiology, School of Medical Sciences, University of Bristol, Bristol, BS8 1TD, United Kingdom.

Cholera is a devastating disease that can reduce a previously healthy individual to the point of death in as little as eight hours. It is caused by Vibrio cholerae that produces a potent cholera enterotoxin responsible for the profuse watery diarrhoea associated with the disease. A similar heat-labile enterotoxin is produced by certain toxinogenic Escherichia coli strains, which cause widespread enteric enteropathies in developing countries and account for up to 70% of cases of 'travellers diarrhoea'. Both cholera toxin (Ctx) and E. coli heat-labile enterotoxin (Etx) are heteroligomeric proteins comprised of an A subunit that possesses ADP-ribosytransferase activity and five B-subunits that mediate binding to receptors, chiefly GM1 ganglioside found ubiquitously on the cell surfaces of mammalian cells. Until relatively recently the receptor binding B-subunits of Ctx and Etx were thought of as inert carrier proteins involved in delivering the toxic A-subunit into target epithelial cells of the gut. However, there is now increasing evidence that the B-subunits possess important 'cell-signalling' properties in their own right, which can exert potent modulatory effects on the immune system. In vitro studies have shown that both CtxB and EtxB influences antigen processing and presentation by macrophages, alters CD4 + T-helper cell differentiation, inhibits release of proinflammatory cytokines by monocytes and triggers CD8 + T-cell apoptosis (Hirst et al. 1998; William et al. 1999). The sum of all these B-subunit mediated effects is to suppress inflammation, which may aid the bacterium in evading the initial host response to infection.

These findings have also led us to test whether the B-subunits can be used to prevent or treat pro-inflammatory autoimmune disorders such as rheumatoid arthritis, diabetes or multiple sclerosis. EtxB was found to be highly efficacious as a therapeutic in animal models of such disorders (Williams et al. 1997). This heralds the possibility the B-subunits might no longer be thought of simply as components of deadly toxins but also as novel immunotherapetics for treatment of human disease.

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BUBONIC PLAGUE IN THE WORLD TODAY

Di Williamson, Biomedical Sciences, DERA Porton Down, Salisbury, United Kingdom

This presentation will describe the incidence of the disease, bubonic plague in the modern world and it's natural history. It will address vaccines past, present and future and evaluate their impact on the prevalence of the disease.

The presentation will describe how understanding of the disease has led to the development of improved vaccines with the isolation of natural virulence factors of the organism and their application to the development of a protective immune response.

TUBERCULOSIS, CURRENT PROBLEMS, FUTURE PROSPECTS

Douglas B Young, Imperial College School of Medicine, Norfolk Place, London, W2 1PG, UK.

Tuberculosis has plagued mankind throughout recorded history, advancing and regressing on different continents over different centuries. Currently, it is estimated that one third of the world's population is exposed to infection with Mycobacterium tuberculosis, resulting in around 8 million new cases and 2-3 million deaths every year. Cases of tuberculosis are increasing as a result of increasing populations, urbanisation, and enhanced susceptibility in association with HIV co-infection.

Is the epidemiology of tuberculosis solely a reflection of changes in social structures, or does adaptation of the pathogen also contribute to changing burdens of disease? The availability of mycobacterial genome sequences and powerful new tools for genotypic analysis, provide an opportunity to begin to analyse the evolution of one of our most successful parasites.

Sequence analysis of individual genes demonstrates a remarkably low level of diversity amongst current isolates of M. tuberculosis. Although point mutations are efficiently selected during development of drug resistance, there is almost no variation in genes encoding housekeeping enzymes or prominent antigens. Significant diversity is generated, however, by insertions and deletions. Distributions of the IS6110 insertion element varies widely amongst clinical isolates. In addition to disruption of genes by insertion, homologous recombination between adjacent IS elements can result in deletion events affecting multiple genetic loci. By studying genotypic variation in the context of contemporary epidemiology, and by analysing M. tuberculosis DNA recovered from bioarchaeological remains, we are currently investigating the possible contribution of pathogen diversity to changing patterns of tuberculosis.

New genetic tools also allow us to investigate the contribution of individual genes to the pathogenesis of M. tuberculosis. Construction of mutant strains by gene replacement provides a powerful new approach to identify targets for novel drugs and to develop strategies for development of improved tuberculosis vaccines.

ANTIBIOTIC RESISTANCE STAPHYLOCOCCUS AUREUS: BACK TO THE FUTURE?

Georgia Duckworth, CDSC, London, UK.

This talk will review the epidemiology and increasing antibiotic resistance of Staphylococcus aureus in the second half of the twentieth century, with a view to assessing what changes may be on the horizon and how developments with a detrimental impact may be averted.

ENTEROCOCCI: OPPORTUNISTS *PAR EXCELLENCE*?

J. Wade, Dulwich Public Health Laboratory & medical Microbiology, Guy's King's & St Thomas' School of Medicine, King's College Hospital, London, SE5 9RS, UK.

The term *entérocoque* and a description of enterococcal endocarditis first appeared in the medical literature in 1899. The role of enterococci in cystitis, and their resistance to antiseptics, heat and desiccation had been described by 1910. Although implicated in serious infections such as septicaemia and endocarditis, enterococci appear devoid of classic pathogenicity mechanisms and are therefore typical opportunists.

At the advent of the modern antimicrobial era enterococci were found to resist killing by sulphonamide and have exhibited relative insusceptability to most subsequent antibacterials. This inherent resistance - especially to cephalosporins - explains the propensity of enterococci to cause superinfection in hospitalised patients receiving the antimicrobials. In the 1980s reports appeared suggesting that the incidence of hospital acquired enterococcal infections - predominantly of urinary tract and blood -was increasing, and outbreaks of hospital cross-infection were described. Enterococci are now a major cause of hospital-acquired infection, with *Enterococcus faecium* more frequently implicated than the more susceptible species, *Enterococcus faecalis*. As progress in medical and surgical management will inevitably generate increasingly susceptible patient groups, the hey-day for opportunists such as the enterococci is probably still to come. Eventually opportunist infections will become the major limiting factor for success in areas such as transplantation, solid tumour therapy and haemato-oncology.

Against this background, and contributing further to the magnitude of the problem, enterococci have acquired mechanisms of resistance to β-lactams, aminoglycosides and glycopeptides (vancomycin and teicoplanin). Glycopeptide-resistant *E. faecium* is now a global problem, causing sporadic infections and outbreaks in high-dependency, renal, liver and other specialist units. Selecting bactericidal antimicrobial regimens for such infections can be difficult - and often impossible.

BACILLUS ANTHRACIS, A BUG WITH ATTITUDE !

Les Baillie, Biomedical Sciences, DERA Porton Down, Salisbury, United Kingdom*

The US Department of Defence describes anthrax spores as the top choice in biological weapons for warfare (www.anthrax.osd.mil/AVIP.htm). They are easy to produce, resistant to most vicissitudes and cause disease via the aerosol route (mortality > 80%). The biology of the organism, its ability to cause disease and the nature of its virulence factors will be described.

Vaccination is the most cost effective form of mass protection. The current US and UK licensed human anthrax vaccines have been in use for many decades and have been shown to be effective in non-human primates. These vaccines where developed using 1950's technology and as a consequence are expense to produce and use a process which is not amenable to large scale production.

Recent advances in biotechnology have enabled researchers to developed improved vaccine expression systems. One such system, based on *Bacillus subtilis* will be described.

What is the future of anthrax vaccines? Access to the genome sequence the organism (www.tigr.org) will enable researchers to better understand the biology of the organism and through this understanding identify new vaccine targets.

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Poster session

Thursday 7th September 2000

The future

NEW VIRAL ENEMIES- FACT OR FICTION

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Emerging infectious diseases are diseases of infectious origin whose incidence in humans has increased within the past two decades or threatens to increase in the near future. Despite historical predictions to the contrary, we remain vulnerable to many new emergent and re-emergent infectious viruses (Meslin 1997).

Nearly all of these emergent virus diseases are zoonotic episodes that involve infectious agents that have involved the transmission of the etiologic agent to humans from an ongoing reservoir life cycle in animals or arthropods, without the permanent establishment of a new life cycle in humans (Murphy 1998). Most frequent factor in emergence is that involving aspects of human behaviour linked to economic impoverishment, war or civil conflict, population growth, migration, tourism or urban decay. Together with environmental changes caused by deforestation/reforestation, changes in water ecosystems, global warming and associated reduction of public health infrastructures, and the probability of virus transfer from endogenous animal hosts to humans' increases (Gubler 1998).

The list of important emergent disease caused by newly discovered arenaviruses, hantaviruses (Glass 1997), filoviruses and paramyxoviruses is impressive and, given what we known about disease ecology, it will only continue to grow. Because there is no way to predict when or where the next important new zoonotic pathogen will emerge or what its ultimate importance might be, investigation at the first sign of emergence of a new zoonotic disease is of particular importance.

How do we respond to new, mutant, or recombinant pathogen strains? What can we anticipate about new major outbreaks? How do we verify outbreaks with the growth of information technology (Grein 2000)? How should we be defending ourselves (MMWR 1998; Heymann and Rodier 1998)? New innovations in the field of prophylaxis, vaccines, understanding of pathogenic phenomena are being produced. Yet the real challenges in pathogen discovery will be the problems associated with sequence interpretation, clinical relevance, and proof of causation. In the end, pathogen discovery will by necessity be a multidisciplinary effort. Only with the co-ordinated interaction of virologists, epidemiologists, pathologists, and clinicians will the role of viruses in disease be clearly defined.

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EVOLUTION OF A BACTERIAL PATHOGEN: MOBILE GENETIC ELEMENTS AND THE EMERGENCE OF PATHOGENIC V. CHOLERAE

B.M. Davis, M.K. Waldor

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The evolution of a number of bacterial pathogens has been significantly shaped by processes for horizontal gene transfer (Ochman et al., 2000). Numerous pathogens utilize virulence genes that apparently were acquired from other organisms, rather than evolved from ancestral loci; consequently, there are often structural similarities among the virulence factors of distinct pathogens, even when these factors are used towards very different ends. Transfer of virulence genes has been mediated by a variety of mobile genetic elements, including plasmids, phages, and transposons, as well as by non-specific processes for DNA transfer, such as transformation. Within contemporary pathogens, some virulence genes are still maintained on mobile elements, while others are now anchored to the chromosome within pathogenicity islands (PAIs).

Horizontal gene transfer has clearly contributed to the emergence of *Vibrio cholerae* as a pathogen. The two most important virulence factors produced by this bacterium - cholera toxin (CT) and toxin coregulated pili (TCP) - were both acquired horizontally: CT-encoding genes lie within the genome of a filamentous phage, CTXϕ (Waldor et al. 1996), while TCP-encoding genes are found within a pathogenicity island, known as the Vibrio pathogenicity island (VPI) (Karaolis et al. 1998). Furthermore, the recently emerged pathogenic O139 strains of V. cholerae, the first non-O1 V. cholerae strains to give rise to cholera epidemics, apparently developed as the result of acquisition of new O antigen-encoding genes by a pathogenic O1 strain (Bik et al. 1995).

CTXϕ - a focus of our work - has several rather unusual features that make it an especially able contributor to transmission of the genes that encode CT, and thus to the evolution of new pathogenic variants of V. cholerae. CTXϕ's replication, secretion, and ongoing evolution will be briefly discussed (Davis et al. 2000; in press).

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PATHOGEN SEQUENCING: THE ROLE OF MALARIA GENOME SEQUENCING IN ANTIMALARIAL DRUG AND VACCINE DEVELOPMENT

Dan Carucci, Director of Genomics and Bioinformatics Malaria Program, Naval Medical Research Institute, Bethesda, US.

The 30 megabase genome of P. falciparum will be completed within the next 2 years, but due to early release of preliminary genome data by the sequencing center, researchers are already capitalizing on these data for the development of novel intervention strategies. Besides identifying potential drug targets through homology searches of known biochemical pathways, research has also begun to characterize entire transcription pathways through the use of techniques such as DNA microarrays and SAGE. For vaccine development, where characterization of protein expression is critical for the synthesis of the optimal vaccine delivery system, high throughput proteomics may provide the best method for antigen discovery. I will discuss the role each of these technologies is playing in our efforts to exploit the malaria genome project data.

GENOME SEQUENCING OF YERSINIA PESTIS

Julian Parkhill, The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.

The Sanger Centre Pathogen Sequencing Unit is currently sequencing the genome of Yersinia pestis CO-92, a biovar Orientalis strain, with funding provided by the Beowulf Genomics initiative of the Wellcome Trust. The chromosome is around 4.7 Mb in size, with three plasmids of 96.2, 70.3 and 9.6 kb. All of the sequence data is publicly available, and the latest sequences can be searched or downloaded at http://www.sanger.ac.uk/Project/Y_pestis. The latest data from the project will be presented. A second strain of Y. pestis is being sequenced at the University of Wisconsin, and genomic sequencing projects for Y. pseudotuberculosis and Y. enterocolitica have begun at Lawrence Livermore National Laboratory and the Sanger Centre respectively. The vast amount of primary and comparative data produced by these related projects will allow new insights into the biology and pathology of these organisms, and inform future therapeutic and prophylactic interventions against their respective diseases.

ANALYSIS OF THE PARTIAL GENOME SEQUENCE OF *FRANCISELLA TULARENSIS* STRAIN SCHU 4

Jan Karlsson[1], Richard G. Prior[2], Kerstin Williams[3], Luther Lindler[4], Katherine A. Brown[5], Nicola Chatwell[2], Karin Hjalmarsson[1], Nick Loman[6], Kerri A. Mack[2], Mark Pallen[7], Michael Popek[4], Gunnar Sandsrtom[1,8], Anders Sjostedt[1,9], Thomas Svensson[1], Ivica Tamas[10], Siv G.E. Andersson[10], Brendan W. Wren[3], Petra C.F. Oyston[2] and Richard W. Titball[2].

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A random library of DNA fragments from a highly virulent strain (Schu 4) of Francisella tularensis has been constructed and partially assembled. The partially assembled data had a G+C content of 33.2%. genes located on plasmids pOM1 and pNFL10, which were isolated from low virulence strains of F. tularensis, were not identified but all of the other known F. tularensis genes were represented once in the assembled data. Genes which could encode enzymes in the shikimate pathway and in the purine biosynthesis and purine salvage pathways were identified. Gene which could encode candidate vaccine antigens have also been identified. This data will be used to develop defined second generation vaccines against F. tularensis, which could be used as replacements for the existing genetically undefined live vaccine strain.

THE SECRETS OF THE *BACILLUS ANTHRACIS* GENOME REVEALED

Timothy Read, The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, Maryland, 20850, USA. Email: tread@tigr.org

Sequencing of a plasmid-cured *Bacillus anthracis* Ames strain is underway using a whole-genome random-shotgun strategy. The initial phase of the project, sequencing a 2-3 kb sheared insert library has been completed and efforts are being directed currently to linking assemblies generated from this library data using both PCR techniques and a large insert library as a 'genomic scaffold'.

Many portions of the *B. anthracis* sequence appear to have similar gene content and organization to the archetypal non-pathogenic *B. subtilis*. At least 60% of *B. anthracis* ORFs have homologues to known *B. subtilis* genes. These include many spore-coat and spore-germination determinants believed to play an important role in virulence. There are many genes without homologues in *B. subtilis* that could be important in anthrax infection, including numerous hemolysins and phospholipases. Also notable was the presence in the genome of numerous copies of a conserved 16 bp palidrome known to regulate expression of extracellular virulence factors in *B. thuringiensis*.

The pXO plasmids that contain the key virulence genes encoding toxin and capsule have recently been sequenced (Okinaka et al., 1999). Intriguingly, although the plasmids appear to have undergone frequent rearrangements, there are few apparent instances of gene transfer between plasmid and chromosome, perhaps suggesting recent arrival of the episome into *B. anthracis*.

The completed sequence will soon be available to the scientific community, however, there are still many secrets to the pathogenesis and origin of this potentially devastating BW agent that will be revealed through functional genomic efforts.

B. anthracis sequence data can be accessed through the TIGR microbial database site (www.tigr.org/tdb/mdb/mdb.html).

Okinaka, R.T. et al..(1999) J. Bacteriol 181:6509-6515

"BIOINFORMATICS: INFORMATION IS POWER OR TOO MUCH KNOWLEDGE IS A DANGEROUS THING ?"

Mark Pallen, Department of Microbiology and Immunology, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BN,UK

Around two dozen procaryotic genomes have been completely sequenced and hundreds more are to follow in the coming decade. This will create a deluge of data, with giga-base-pairs of sequence and hundreds of thousands of new genes, many previously unknown to science. Bioinformatics promises to provide the tools to store, analyse, inter-link and make sense of this data, priming new hypothesis-driven research and delivering new drug, vaccine and diagnostic targets. However, there is a risk that the data flood will overwhelm the capacity of the scientific community to cope.

USING PROTEOMICS TO EXPLORE PATHOGEN SURVIVAL RESPONSES

David O'Connor, Division of Biochemistry & Molecular Biology, School of Biological Sciences, University of Southampton SO16 7PX, UK

Proteins are the ultimate cellular effectors of essentially all cellular processes and constitute the vast majority of drug and vaccine targets. Thus, the systematic analysis of proteins and in particular their pattern of expression, interactions and modifications- proteomics- is becoming an increasingly important tool for defining their biological functions in the post-genomic era. Microbial pathogens present special opportunities for proteomic analysis that are not available for other types of organisms, due mainly to the relative abundance of information on their genomes, their low levels of functional redundancy and their experimental tractability. They are being used to develop and validate powerful new experimental approaches that surmount some important current limitations in this field.

The talk will focus on the identification and regulation of overlapping sets of proteins involved in the survival of several pathogens, including *Mycobacterium tuberculosis*.. The usefulness of proteomics at different stages of research investigation will be illustrated by reference to the discovery and characterisation of BipA, a novel regulatory GTPase that controls a range of virulence-related processes in several pathogens. The challenge now is to provide accurate biological explanations for the complex and sometimes enigmatic responses observed at the proteomic level. Ultimately, this information will provide an integrated and deeper understanding of the survival strategies used by dangerous pathogens.

MICROBIAL GENOMICS - A REVOLUTION IN ANTIMICROBIAL DRUG-HUNTING

A.E.Allsop, J.Rosamond & R.Illingworth Astra Zeneca, Alderley House, Alderley Park, Macclesfield, Cheshire, UK

Over the past 40 years the search for new antibiotics has been largely restricted to well known compound classes active against a standard set of drug targets. Although many effective compounds have been discovered, insufficient chemical variability has been generated to prevent a serious escalation in clinical resistance. Recent advances in genomics have provided an opportunity to expand the range of potential drug targets and facilitated a fundamental shift from direct anti-microbial screening programmes towards rational target-based strategies. Target selection can in this way be enhanced by data comparison at the genome level, enabling the best possible choice, based on desired properties of potential agents in the clinic, such as microbiological spectrum and selectivity. Following target selection, a rational approach enables "designer" chemistry in the optimisation of interaction between compound and target at the molecular level.

No matter how well informed the choice of target, there are still many challenges remaining in the search for new antibiotics. The discovery and optimisation of compounds which fulfil clinical demands using the advantages of rational, molecular strategies rely on the ability to ensure that target interaction and antimicrobial activity are linked. It is only recently that "second generation" genomics technologies have become available to investigate the response of an entire microbial cell to challenge with a drug-candidate. In this way the application of genome-based technologies such as expression profiling and proteomics will lead to further changes in the drug discovery paradigm by combining the strengths and advantages of target-based approaches with investigation of antimicrobial activity.

Antibiotics of the future will still incur resistance. However with a variety of new mechanisms in use and more specific spectra, the hope is to create sufficient choice in the clinic to prevent reliance on a small number of drug-classes.

ARE ANTIVIRAL AGENTS A REALISTIC OPTION IN CONTROL OF DANGEROUS VIRUSES ? COST AND RISK VERSUS BENEFIT

C.R.Penn Centre for Applied Microbiology and Research (CAMR), Porton Down, Salisbury,SP4 OJG,UK

Prospects for development of new antiviral agents for the treatment of dangerous virus infections will be reviewed, with examples of recent successes and failures, considering three factors that strongly influence the success of development of new antiviral agents.

The high cost of development of any new human therapeutic agent, combined with the high risk of failure during development (such that the total cost of a pharmaceutical company's R&D programme must be met by the return on successful products), mean that industry is normally only prepared to undertake a development programme when the target disease is prevalent in wealthier parts of the world. HIV, influenza and hepatitis B viruses represent examples where the prevalence of disease worldwide has resulted in significant investment such that effective antiviral agents are now available. This is not the case for other dangerous viruses such as the haemorrhagic fever viruses, where infections occur rarely, or mostly in developing countries.

The above limitation could be overcome by the development of broad spectrum antiviral agents. However, unlike bacteria, different virus families have little (if any) metabolic or molecular processes in common, and depend on host cell processes for much of their replication. Agents that appear to have broad spectrum antiviral activity often act at least in part through inhibition of host cell metabolism (e.g. ribavirin) and in many cases prove too toxic for use (e.g. fialauridine). The most notable success of a broad spectrum antiviral agent is lamivudine, which inhibits the reverse transcriptase of both HIV and hepatitis B viruses, and has proved to be safe and effective.

The third factor to consider is the role of virus replication in the disease process. In chronic infections, where persistent virus replication drives disease, an antiviral agent can result in significant clinical benefit. In other acute infections, where severe disease is primarily immune response driven (leading in the most severe cases to shock) antiviral agents may be less effective unless used early in the course of infection, or as prophylaxis.

EMERGING VACCINES TECHNOLOGIES: EXCITING PROSPECTS FOR NEW PRODUCTS

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Vaccination has proved to be an extremely effective tool in the prevention of infectious diseases. The control of diseases such as tetanus, diphtheria, whooping cough, polio and smallpox have largely been achieved with 'traditional vaccines' i.e. those that derived in the pre-molecular genetics era such as chemically inactivated toxins, simple killed whole cell preparations of the pathogen or live attenuated vaccines that were derived empirically. Although effective, such technologies have not been able to deal with the wide spectrum of bacterial and viral disease.

However, these successes, coupled with the emergence of many multi-antibiotic resistant strains of bacteria has re-awakened the interest in vaccines. This has encouraged and enormous amount of activity in the research community aimed at developing better defined, more efficacious vaccines to replace existing products and also to find ways of controlling diseases for which up to now, there has been no effective prophylaxis.

The renewed effort in vaccine research and development has been facilitated by the 'Biotechnology revolution'. The advent of molecular biological techniques, a greater understanding of immune responses required to elicit immunity coupled with a real lreap forward in production technologies and delivery systems has led to the identification of many potential targets for vaccination.

PREVENTING DELIBERATE DISEASE: FUTURE CONTROL REGIMES

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The use of disease to attack humans, animals and plants is totally prohibited by the Biological and Toxins Weapons Convention (BTWC) which was opened for signature in 1972 and entered into force in 1975. This convention now has 143 States parties and 18 signatory provisions for verification or the monitoring of compliance. The past two decades has seen increased concern about the possible misuse of advances in microbiology and biotechnology as well as about the increased number of states that either have or may be seeking biological weapons. Consequently, steps have been taken to negotiate a Protocol to strengthen the effectiveness and improve the implementation of the BTWC. These negotiations are nearing completion and will require declarations, declaration follow-up procedures and investigations of possible non-compliance concerns as well as including provisions to promote international scientific and technical exchange and co-operation. The importance is recognised for international security and prosperity of a web of reassurance comprising comprehensive prohibitions, effective national controls both within countries and of transfer between countries, broad band preparedness for outbreaks of disease, whether natural or deliberate, and determined responses to a breach or threatened breach of the prohibition. The implications of the BTWC protocol and of the Cartagena Biosafety Protocol for work involving dangerous pathogens will be examined from the point of view of countries within the European Union and around the world. The benefits that accrue from increasing transparency and building confidence through appropriate public disclosure and national infrastructure monitoring of the work with dangerous pathogens will be examined.

ABSTRACTS

METACYCLOGENEIS OF *LEISHMANIA MAJOR* IN ACIDIC MEDIUM

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Acidic medium was used to stimulate the production of a homogenous stationary phase promastigotes, morphologically and functionally similar to the in vivo infective form. They possessed a short (< 8 Um) and narrow (< 1.5 Um) cell body with a flagellum twice or more its length. They were PNA, highly infective to peritoneal macrophages in vitro and expressed high phosphatase activity. By elevating the incubating temperature, almost all of these promastigotes were transformed to amastigotes. This provides a successful method for pure axenic culture of metacyclics as well as amastigotes, which may help greatly in understanding the pathogenesis and molecular biology of *Leishmania*.

STUDIES ON A NEWLY EMERGING PROTOZOAL PATHOGEN: CYCLOSPORA CAYETANENSIS

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Cyclospora is frequently misdiagnosed with *Cryptosporidia*. Stool samples were collected from 150 immunocompromised patients and concentrated by the parasep faecal parasite concentration and the discontinuous percoll gradient methods. Wet mount examination was done and the parasite was successfully stained with modified Ziehl Neelson (ZN) and safranin methylene blue stain. *Cyclospora* was detected in 4% of cases examined. *Cyclospora* was easily differentiated from *Cryptosporidia* by using the modified detergent ZN stain whereby *Cyclospora* resist staining and *Cryptosporidia* pick up the pink colouration. Scanning and transmission electron microscopic examinations were done to the unsporualated *Cyclospora* oocysts. They appeared as spherical objects with an outer fibrillar coat, an indentation and sutures. These spherical objects also contained light and dark granules. In studying the possible sources of transmission of this parasite, sporulated and unsporulated oocysts were detected in tap water and lettuce heads, which support the theory that water and food could be the sources of transmission of this parasite.

FINDINGS OF *Y. PESTIS* CAPSULAR PROTEIN ADHESION FUNCTION

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Yop virulon or the type III secretion system in *Y. pestis, Y. pseudotuberculosis* and *Y. enterocolitica* enabled the protection of bacterial cells from macrophages suppressing their phagocytic and signal functions. Using the type III-secretion system, Yersinia cells injected the toxic bacterial proteins into macrophage cytoplasm through the Ysc secretion channel acting like a biological syringe. Yop delivery needed the contact of bacteria with macrophages. Protein YadA enabled adhesion and translocation in *Y. pseudotuberculosis and Y. enterocolitica. Y. pestis* lacked that protein. According to the data obtained, Caf1 anchored on the bacterial cell surface could play the role of *Y. pestis* adhesion. Mice fibroblasts NIH3T3 expressing constitutively receptors to IL-1 β and IL-1α, had been taken for the studies. Specific receptors to rHuIL-1β ($K_d=10^{-10}$M), rHuIL-1α ($K_d=10^{-10}$M) and Caf1 ($K_d=10^{-10}$M) were detected on the fibroblast surface. Caf1 inhibited affinity binding of labeled rHuIL-1β, but not rHuIL-1α with NIH3T3 cells. In the presence of Caf1, specific binding of the labeled rHuIL-1β was characterized by $K_d=10^{-9}$M. Thus, rHuIL-1β in the presence of Caf1 lost the ability to interact with high affinity binding sites and bound with medium affinity sites. Specific receptors to *Y. pestis* Caf1 were also found on the surface of human macrophage cell line U-937. The interaction of Caf1 with IL-1β receptor on the macrophage could play an important role in the fatal human pneumonic plague pathogenesis. In vivo capsule formation occurred in human lungs after aerosol infection.

DETECTION OF ENTEROPATHOGENIC *YERSINIA ENTEROCOLITICA* FROM CONTAMINATED WATER

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Enteropathogenic *Yersinia enterocolitica* was isolated from contaminated water by the traditional culture techniques. Water samples were filtered (Millipore) and filters were enriched over night in a Yersinia Selective enrichment broth and subsequently on Yersinia CIN agar at 37^0C for 24hrs. Complete study of the phenotypic and biochemical characteristics of the isolated strain was conducted. The characteristics of virulence such as autoagglutination at 37^0C, calcium dependent growth at 37^0C and uptake of Congo red dye were also studied. Finally the identification was carried out by a Latex agglutination test (Microgen) for the detection of enteropathogenic Yersinia enterocolitica (Myf).

THE HUMAN PLAGUE IN KAZAKHSTAN

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On 39% of all territory of Kazakhstan there are the natural foci of a plague. Annually epizooties with isolation of hundreds Yersinia pestis strains are registered from the rodents and fleas. From 1904 to 1999 more than 400 human plague outbreaks are determined. Its were connected with wild rodents and their fleas, ill camels, hunting on the jackrabbits, foxes, saiga contamination. The epidemic spreading was watched up to 1949, and from the indicated time the contamination has sporadic nature (on the average one ill patient annually) due to creation of a specialised antiplague service, which consists of research institute, antiplague stations and its local departments.

Usage for treatment of the new scheme has allowed sharply to lower plague lethality. The scheme includes treatment with intensive desitoxication, introducing bacteriostatic drugs, and only for the second day of bactericidal antibiotics. It is necessary a usage of reserve antibiotics if patient man have treat with hormones, which provocate development of a bacterial resistance to used antibiotic.

But last years the epidemic potential of the natural foci was increased because of realisation in its territory of prospecting and output oil activities and the contact of the person to wilderness was increased. In countrysides contamination of people housing by fleas-vectors of a plague is registered. The present reduction of social condition in Kazakhstan decreases a resistance of the people to infection diseases. The liquidation of village public health services deprives the population of a well-timed medical care. The volume of preventive actions spent by an antiplague service was reduced. Its and also anomalously high activity of the natural foci was resulted in a contamination in 1999 nine persons, two of which have died. One patient have died in result of a late rendering of a medical care, and second patient – from peaking chronic diseases of heart and nephroses. The father and brother was infected from an ill boy with a transabdominal syndrome of plague, which accompanied with him in a train.

The plague microbe was isolated from throat probes of contact men, but as a result of well-timed preventive treatment they were not ill.

Increased migratory activity of the population introduce danger of plague 13 spreading, that is an actual problem modern epidemiological surveillance.

STUDY OF NEW DIAGNOSTIC TEST SYSTEM FOR BRUCELLA SPP. DETECTION USING PCR

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The diagnostic value of new test system for Brucella spp. detection by PCR was studied. It consists of two primer pairs, specific to the outer membrane protein of Brucella 31 kDa, and a set of reagents for nested reaction performance.

It was shown, that test system under study was characterised by high sensitivity as far as *B. abortus*, *B. melitensis, B. suis, B. ovis, B. rangiferi*, were concerned, specificity and reproducibility. All in all 317 samples were analysed. Using this test system 18,7% of samples containing Brucella at concentration of 10 mc/ml, 84,4% of samples containing 100 mc/ml and 100% of samples containing 1.10^3 mc/ml were detected. While analysing samples, infected with *Y. enterocolitica, V. cholerae and F. tularensis*, at a dose of 1.10^7 mc/ml, no positive results were registered. Reproducibility of PCR results using test system under study with 10 positive samples containing *B. abortus* and 10 negative samples containing *Y. enterocolitica*, was 100%. Analysis of environmental objects and biological materials artificially infected with Brucella at concentration of 1.10^3 mc/ml resulted in 100% detection of positive samples. During the study of organs (lymphatic node, blood, brain, spleen, liver) of guinea pigs immunised with *B. abortus* at a dose of 2.10^9 mc, and 30 days after infected with 20 mc of *B. melitensis* 565, PCR analysis, allowed detection of brucellosis agent in 5 of 12 animals (in 6 of 60 samples).

Comparative trial of test system during the study of blood from chronic brucellosis patients showed higher diagnostic value of the test system as compared to serological methods (ELISA, RPHA, RH, RA) and skin test, as regards informative potential (frequency of detection) and possibility to receive analysis results quickly. The advantage of PCR method was shown, in comparison to nutrient medium (comparison preparation), as regards Brucella detection in environmental objects and possibility to receive analysis results quickly. The test system is recommended for application by practical health services.

SIGNATURE-TAGGED MUTAGENESIS OF *BURKHOLDERIA PSEUDOMALLEI*

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Burkolderia pseudomallei occurs in tropical and subtropical climates and is the causative agent of melioidosis. Melioidosis used to be considered a relatively rare disease, but with improved diagnostic techniques and an increase in global travel, melioidosis is becoming more common and being isolated from more varied environments.

Signature-tagged mutagenesis has been used to study the genetic elements necessary for virulence in a wide range of pathogens. In this study signature-tagged mutagenesis was used to examine the virulence determinants of the pathogen *B. pseudomallei.* The technique relies on a pool of 96 plasmid DNAs each carrying a transposon tagged with a unique nucleotide tag. Recovery of this tag by polymerase chain reaction (PCR) allows identification of each transposon and hence each mutant. Integration of the transposon within the genome of *B. pseudomallei* was examined. Animals were inoculated with a pool of 96 transposon mutants. Organisms were recovered from infected animals and the tags present in the recovery pool of bacteria were compared to those present in the pool of bacteria used to inoculate the mice. By this method mutants putatively attenuated for virulence were identified. Selected mutants were then characterised *in vitro* and *in vivo.*

THE MAIN RESULTS OF STUDY OF THE NATURAL PLAGUE FOCI IN CENTRAL ASIA AND KAZAKHSTAN

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On Central Asia and Kazakhstan territories there are different natural plague foci – marmot, souslik, gerbil and vole.

It is established, that parasitic system causing natural plague focus, is complex biocaenosis structure. Functions of the biological plague hosts fulfil in an equal measure both homothermic carriers , and piokilothemic carriers. The character of mutual relations of the agent with homothermic host and ectoparasitic-carrier has developed as a result of long evolutionary development and grows out adaptation of the agent to existence in certain environment conditions on the ground of topical connection.

Study of spatial structure have shown, that the territory of any natural plague focus in epizootological relation is non-uniform. The separate parts of the focus can will be distinguished from each other on the sizes, features of a landscape, frequency, duration and epizootic intensity.

The analysis and generalisation of these data have allowed to differentiate spatial structure of the focus on morphological parts: sites of steady infection preservation, infection carrying out, and free from the plague microbe. The establishment of temporary and steady spatially expressed epizootic of structures in settlements makes a basis of practical measures which are directed on a substantiation and optimisation of epizootologic methods of investigation to forecast epizootic process in concrete territory.

It is established, that the growing influence of anthropogenous factors results in the certain transformation of the natural plague foci. Thus anthropogenous loading in one cases causes pessimistic, and in others – optimum conditions for existence of carriers and plague microbe. The study of diverse influences of economic activity of the man in the natural plague foci and the analysis of the socio-economic characteristic of Central Asia and Kazakhstan has allowed to carry out typing of anthropogenous influences on a landscape, which in turn determines necessity of differential realisation of epidemiologic surveillance in these regions.

ANTIBIOTIC RESISTANCE IN AN *IN VITRO* GUT SYSTEM

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In vitro gut models have been produced for many stages of the gastrointestinal tract with variations for different animal species. Generally these have been run under anaerobic conditions, but the intestine, and the small intestine in particular, is actually a complex environment of both anaerobic and aerobic conditions. Accordingly, a model pig ileum was developed with a controlled oxygen supply. The model was then validated as comparable with the porcine small intestine in terms of specific and general microbial population, oxygen and VFA content.

The effect of the antibiotic tetracycline (Te) on the *Escherichia coli* population of the porcine ileum was investigated using this *in vitro* model. Feeding sub-therapeutic (22 mg Te Kg^{-1} diet) levels of Te over a period of 10 d produced a gradual increase in the proportion of *E. coli* resistant to Te, whilst the addition of a therapeutic dose (150 mg Te Kg^{-1} diet) for 48 h accelerated resistance development. Subsequent antibiotic withdrawal resulted in no significant decline ($P>0.05$) over time (16 d) in the proportion of Te resistant *E. coli*.

The addition of four daily challenges to this established resistant population with a faecal suspension of *E. coli* (1.5% of the model's total *E. coli* population) from an animal gut populated by predominantly sensitive bacteria, produced a dramatic reduction in the proportion of *E. coli* resistant to Te ($P<0.01$). Similarly a single dose of a nutrient broth incubated faecal suspension of largely susceptible *E. coli* brought about a significant ($P<0.05$) decline in *E. coli* Te resistance. However, the effect could not be replicated with pure *E. coli* cultures from the same susceptible population, indicating that more than just the susceptible *E. coli* strains were required to displace the resident resistant population. Important co-factors in the faecal suspension may include other coliforms, bacteria or possibly phages.

THE DETECTION AND ENUMERATION OF STRESSED *ESCHERICHIA COLI* O157 ENVIRONMENTAL SAMPLES

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There is currently much concern regarding the public health hazard presented by *Escherichia coli* O157 in food, agriculture and waste management systems. Most of these systems exert environmental stressors that may adversely affect the subsequent detection and enumeration of *E. coli* O157. At present most methods available either resuscitate stress cells, but cannot enumerate (e.g. presence/absence tests) enumerate but cannot resuscitate (e.g. MPN methods) or merely indicate the presence of relatively large numbers of cells (immunological methods).

Here we describe a method that allows both the resuscitation of environmentally stressed cells, and also permits their enumeration. The method is applicable to a wide variety of sample types and performs well with high background microbial loading. It relies upon simple, readily available equipment and media ,does not require extensive operator training and allows evaluation with 24hrs.

ASSEMBLY PATHWAY OF THE F1 CAPSULE OF *YERSINIA PESTIS*

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Yersinia pestis is the causative agent of plague and remains endemic in many countries throughout the world, where it continues to cause isolated outbreaks of infection among humans. Shortly after infection of the mammalian host, *Y. pestis* synthesises a large, amorphous capsule (the F1 antigen) on the cell surface. The capsule is compromised of a high molecular weight polymer of a single 15.5 kDa polypeptide, Caf1, which is a strong immunogen and is antiphagocytic, but does not appear to be essential for virulence in mouse models. The subunit is encoded on a 101 kb plasmid in an operon together with two genes required for capsule secretion and assembly: *caf1M*, which encodes a periplasmic chaperone and *caf1A*, which encodes an outer membrane secretin.

Caf1 is a key component of both older whole cell and newer subunit anti-plague vaccines. Yet little is known about the structure and folding pathway of the F1 capsule. The pathway bears homology to the assembly of Pap pili in uropathogenic *Escherichia coli*, however, the assembly components of the F1 capsule belong to a much less well-studied subfamily which assemble simple surface structures rather than complex pili. A number of distinguishing features identify the chaperone of this subfamily: (i) a long variable sequence (FGL) between the designated F1 and G1 – strands (ii) an N-terminal extension and (iii) a disulphide bond adjacent to the subunit binding site. Proximity of each of these features to the subunit binding site, suggests that they may have an important influence on subunit binding, folding and polymerisation. This is being investigated in an *E. coli* model system. Formation of the disulphide bond was shown to be essential to *in vivo* folding of Caf1M, but was not essential for subunit binding or export. Analyses of deletion derivatives demonstrated that the FGL sequence is required for stable chaperone: subunit interaction, but does not contribute significantly to the stability of the final conformation of Caf1M. Mutagenesis of the FGL sequence identified V126 and V128 as key residues in subunit binding. Possession of similar hydrophobic residues by other members of this subfamily suggests that this region may contribute to specificity and high affinity subunit binding during formation of these simple surface structures. Evidence that Caf1M influences folding and polymerisation of Caf1 subunit was also obtained.

Following complex formation the folded Caf1 subunit is targeted to the outer membrane Caf1A protein for surface localisation. Analyses of solubilised Caf1A by sucrose density centrifugation indicate that this protein forms an oligomer consistent with its function as an outer membrane secretin.

PROTEOMIC ANALYSIS OF *FRANCISELLA TULARENSIS*

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Type-A strains of *Francisella tularensis* are extremely virulent in man whereas type-B strains have reduced virulence. The underlying reasons for this difference remains unknown. Currently work is proceeding to determine whether this can be related to differences between the proteomes of the two biovars. A procedure was developed that allows pathogen sample preparation under containment conditions and subsequent proteome analysis using two-dimensional gel electrophoresis. A comparison of strains Schu4 (type-A) and LVS (type-B), prepared under identical conditions, was made. Results showed a number of different proteins and differences in the level of protein expression between the two strains. Gels with high protein loading were electro-blotted onto PVDF membrane. Protein spots were cut from the membrane and characterised by N-terminal sequencing and database search. Proteins GroEL and GroES were identified in LVS and Schu4. Work is currently underway to characterise certain strain-unique proteins.

CRYPTOSPORIDIUM PARVUM AN EMERGENT ENTERAL PATHOGEN

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Background

In the last two decades many so-called "new" intestinal parasitic protozoa were recognised as potential and actual human pathogens such as the spore-forming *Cryptosporidium* (C.), microsporidia, *Isopora* and *Cyclospora*, *Babesia* (infecting human erythrocytes) and *Naegleria* (which cause a form of meningoencephalitis). Data on prevalence of these infections are not available in a uniform manner.

The aim of our study was to determine the prevalence of *C. parvum* oocysts in children with symptoms of gastro-enteritis.

Material and methods

Stool specimens of 412 children who attended paediatric and general practices and 117 children from a control group were investigated for the presence of *C. parvum* oocysts. The modified Zielhl-Neelson and Giemsa staining were used.

Results

C. parvum oocysts were present in 16 (3.8%) of children with diarrheal disease and in none from the control group 4 (25%) of cryptosporoodiosis cases were infected with bacterial pathogens (*C. jejuni*, *E. coli* enteropathogen, *Aeromanas caviae*), too. The fecal samples from 7 (43.7%) children were massive infected while in 9 (56.2%) children the specimens were low infected; there are no differences in clinical symptoms between these two groups.

Conclusions

The results of our study suggest that *C. parvum* is a common enteric pathogen for the children in our area. Because the oocysts have the ability to survive in soil for many months and the transmission to humans is possible via food, water and directly from infected to non-infected persons, it is necessary a better surveillance of this emerging disease.

IN VITRO ACTIVITY OF NORFLOXACIN AND CIPROFLOXACIN AGAINST GRAM-NEGATIVE BACILLI ISOLATED IN NORD-EAST OF ROMANIA

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Objective

To evaluate the influence of an increasing consumption of quinolones on the prevalence of quinolone resistance in clinical and communitary bacterial isolates.

Material and methods

There were tested a total of 342 Gram-negative bacilli strains belonging to *Enterobacteriaceae* family, *P. aeruginosa* and *Acinetobacter Iwoffi* species. The strains were isolated between January 1998 – March 2000 from acute diarrhoea and urinary tract infections (UTI) from hospitalised and from health units patients, children and adults. The susceptibility tests against norfloxacin (NOR) and ciprofloxacin (CIP) were done by disk diffusion method (Kirby-Bauer), using the interpretative criteria recommended by NCCLS, 1997.

Results

12 (3.5%) of all strains were resistant to NOR and 10 (2.9%) to CIP. The hospital isolates presented the highest resistance percents 6.0% to NOR and 4.5% to CIP, comparative with communitary isolates: 1.9% to NOR and CIP. 5.5% and 4.5% of strains isolated from urine and 2.6% and 2.1% of strains isolated from faeces were resistant to NOR and CIP. The hospital isolates from patients with UTI and the strains isolates from outpatients which subsequently developed on UTI had the highest resistance percent 9.7% to NOR and 7.3% to CIP comparative strains – 2.9% resistance to both quinolones. Many resistant *E. coli* and *P. aeruginosa* strains were isolated from recurrent UTI treated with a lot of antibodies, including quinolones. *P. aeruginosa*, *E. coli*, *Klebsiella* spp, *Enterobacter chloacae*, *Proteus* spp and *Salmonella* strains were frequently resistant presenting ≥ 8-10 resistance determinants.

Conclusions

The frequency of resistant Gram-negative bacilli varied between 1.9 percent to NOR as well as for CIP in communitary isolates and 9.7% respectively 7.3% in the hospitals strains. The uncontrolled and incorrect usage of the quinolones facilitates the spread of the resistant strains.

IDENTIFICATION OF CHANGES IN GENE EXPRESSION INDUCED BY TOXIC AGENTS: IMPLICATIONS FOR THERAPY AND RAPID DIAGNOSIS

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Our studies have been directed towards creating a global library of host gene expression responses to anthrax, botulinum, brucella, plague, cholera and other staphylococcal enterotoxins (SE). Since gene expression changes are early responses to these biological threat agents, this approach permits determination of host functional responses prior to the onset of symptoms or illness. This approach is especially important since a) genetically altered agents could be unidentifiable by conventional structural-based probes, b) potentiating agents at undetectable levels can make biological threat agents significantly more toxic and difficult to treat, and c) treatment could be tailored to the needs of the patient. The ultimate objective is to utilize peripheral blood lymphoid cells from exposed individuals since these cells are a readily accessible reservoir of historical information.

We are systematically examining lymphoid cells exposed to these toxic agents using complementary methods, gene arrays and differential display, to screen for alterations in gene expression.

Currently we have observed that some genes respond to multiple biological threat agents, while others may be specific for a particular toxin. In the case of the shock-inducing toxins, we also detected some common and major changes in expression of gene involved in regulation of vascular tone, a hallmark characteristic of impending lethal shock. In order to validate the *in vitro* findings, monkeys were exposed to SEB by aerosol, PBMC were obtained, RNA isolated and gene array analysis performed. By 30 mins post-exposure and prior to onset of symptoms, the gene pattern indicated the toxin identity and that the exposure dose was mild to moderate. An ancillary benefit is that gene changes induced by toxic agents, provides the basis for design of new intervention strategies to prevent or ameliorate severe illness.

EARLY *BACILLUS ANTHRACIS* – MACROPHAGE INTERACTIONS: INTRACELLULAR SURVIVAL AND ESCAPE

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This study describes early, intracellular events occurring during the establishment phase of *Bacillus anthracis* infections. Anthrax infections are initiated by dormant endospores gaining access to the mammalian host and becoming engulfed by regional macrophages (Mϕ). During systemic anthrax, late stage events include vegetative growth in the blood to very high titres and the release of the anthrax toxin complex, which causes disease symptoms and death. This study focuses on the early events occurring during the first few hours of the *B. anthracis* infectious cycle, from endospore germination up to and including release of the vegetative cell from phagocytes. We found that newly vegetative bacilli escaped from the phagocytic vesicles of cultured Mϕ and replicated within the cytoplasm of these cells. Release from the Mϕ occurred 4-6 hours after endospore phagocytosis, timing that correlates with anthrax infection of test animals. Genetic analysis from this study indicates that the toxin plasmid pX01 is required for release from the Mϕ, while the capsule plasmid pX02 is not. The transactivator *atxA*, located on pX01, was also found to be essential for release, but the toxin genes themselves were not required. This suggests that Mϕ-release of anthrax bacilli, like its other known virulence factors, may be *atxA*-regulated. The putative "escape" genes may be located on the chromosome and/or on pX01.

EARLY *BACILLUS ANTHRACIS* – MACROPHAGE INTERACTIONS: INTRACELLULAR SURVIVAL AND ESCAPE

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MYCOBACTERIUM TUBERCULOSIS DNA FROM 18TH – 19TH CENTURY HUNGARIANS

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Tuberculosis was endemic in Europe in the 18th – 19th centuries so examination of human material from this period should detect Mycobacterium tuberculosis DNA and enable comparison with modern isolates. The remains from 265 individuals were discovered in the sealed crypts of the Dominican Church of Vδc, Hungary in 1994-1995, during building work. They were of a middle-class population dating from 1731-1838. Many contemporary written records are available giving date of death, age, sex, family name, relatives and sometimes the cause of death. Several bodies can be placed into family groups and categorised as of German or Hungarian ethnic origin. This information, combined with Mycobacterium tuberculosis complex (MTB) DNA sequencing data and molecular fingerprinting techniques, can be used to determine the epidemiology of TB infection in this community. Samples were collected from 174 individuals. Many were naturally mummified so soft tissues were available. Ribs were sampled from skeletelised material. Using a specific PCR for MTB DNA, 38% of the individuals were positive. Nested PCR based on a MTB-specific fragment of IS6110 with a product of 92bp was the most sensitive method. Some samples were well-preserved and were positive for several different MTB-specific target sequences. These better-preserved specimens were examined for silent point mutations in codon 463 of katG and codon 95 of gyrA. Sequencing of samples has placed these strains into genotypic groups 2 or 3 (Sreevatsan et al, PNAS 94: 9869-74, 1997). Point mutations associated with drug resistance in the rpoB, katG, inhA and 23S rRNA genes are also being screened. An initial attempt at molecular fingerprinting based on repetitive spacer regions (spoligotyping) has demonstrated the same spoligotype within a family group. This work indicates it is possible to extend the timeframe for the examination of molecular fingerprints which may throw light on how these change over time.

CARRIAGE OF ANTIBIOTIC RESISTANT *ESCHERICHIA COLI* IN PIGS AND SPREAD TO PROGENY AND THE FOOD CHAIN

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Numbers of total *E. coli* and *E. coli* resistant to the antibiotics tetracycline (T), ampicillin (A), nalidixic acid, (NA) and apramycin (Apr) were monitored in pig faecal samples and pork products. Total *E. coli* counts were performed on MacConkey agar, resistant strains were enumerated on the same media supplemented with the appropriate antibiotic T, 16μg ml^{-1}, A, 16μg ml^{-1}; NA, 15μg ml^{-1} and Apr, 32μg ml^{-1}.

E. coli resistant to tetracycline and ampicillin were found in all intensively reared pigs, making an average of 41% and 15% of the total *E. coli* population in these animals respectively. Similar levels of resistance were found among pigs at lairage prior to slaughter. Resistance to nalidixic acid and apramycin was much lower, 3% or less and more variable between farms.

Pigs from a variety of farms reared on an exclusively antibiotic free diet were found to carry a significant antibiotic resistant *E. coli* population. Tetracycline and ampicillin resistant *E. coli*, were almost always present, though nalidixic acid and apramycin resistant *E. coli* were present only at extremely low or non-detectable levels. However, there was a significant reduction in the proportion of resistant *E. coli* in the antibiotic-free animals, with tetracycline and ampicillin levels at 6% and 2% of the total population, compared to 41% and 15% respectively in the intensive animals.

It is believed that resistance can persist over many generations following the withdrawal of antibiotic usage. In this study it was found that the carriage of antibiotic resistant *E. coli* in pre-weaning piglets was significantly correlated with the resistance pattern of the sow. Indicating maternal transfer aids the establishment of a resistant microflora at an early stage in life.

Pork products, sausages, mince and chops were tested for the presence of *E. coli*. Most (72%) had levels below $100g^{-1}$, 60% less than $10g^{-1}$. Resistant *E. coli* were detected but only after culturing enrichment broths of 0.1-1.0g samples of the product. Patterns of resistance, ie principally to tetracycline and ampicillin, suggest that these originate from the gut. However, the extremely low incidence of antibiotic resistant *E. coli* in pork products indicates hygiene has a significant role in breaking the link between farm and consumer.

MONOCLONAL ANTIBODIES TO SOME SEROGROUP-SPECIFIC PROTEINS OF YERSINIA PSEUDOTUBERCULOSIS

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A library of monoclonal antibodies (Mabs) was generated by immunisation of BALB/c mice with Yersinia pseudotuberculosis II serogroup whole cells. Mabs of the cloned hybridomas recognised Y. pseudotuberculosis belonging to different serogroups: 3.13% - I; 4.16% - II; 10.41% - III; 3.13% - IV; 10.47% - V; 1.04% - VI serogroup (the bacteria were cultivated at 285°C), and 7.29% - II; 1.04% - II; 2.08% - IV serogroup (the bacteria were cultivated at 375°C). No cross-reaction with other pathogenic yersiniae was observed. All the Mabs (except 2A2) reacted only with Y. pseudotuberculosis whole cells but not with proteinase K-digested ones. This strongly suggested that most Mabs obtained were directed to protein epitopes.

In immunoblotting Mab 3A2 to Y. pseudotuberculosis I (28 degrees (d) C) reacted with 2 proteins of mw. 34 kDa and 38 kDa and 2 carbonhdyrate components of 20 kDa and 38 kDa. Mab 3C3 to Y. pseudotuberculosis II (28 d) reacted with 2 proteins of 34.6 kDa and 45.7 kDa; Mab 1D3 to Y. pseudotuberculosis II (37 d) reacted with the proteins of 45.7 kDa and 24.5 kDa. Mab 2B4 to Y. pseudotuberculosis IV (28 d) reacted with 36.6 kDa protein. The data probably lead to the conclusion that intact bacterial cell of Y. pseudotuberculosis produces serologically active protein antigens bearing specific epitopes of at least 6 Y. pseudotuberculosis serogroups.

SEROLOGICAL RELATIONSHIP OF *E. COLI* CFA1 AND PROTEINS OF SOME ENTEROPATHOGENIC BACTERIA IS ESTABLISHED

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Antibodies to colonisation fimbrial antigen 1 (CFA1) isolated from *E. coli* J53 harbouring pCFA1 according to Evans et al (1979) were raised in BALB/c mice. The anti-CFA1 antibodies (anti-CFA1-ab) reacted with the original antigen in the titre 1:5000 in ELISA and cross-reacted with a wide spectrum of enteropathogenic bacteria. The anti-CFA1-ab obtained were used in immunoblotting of electrophoresed whole-cell lysates of Vibrio cholerae 01 serogroup – 569B Inaba, M-35 Ogawa; 0139 serogroup – M045 and P16064 strains, Yersinia pseudotuberculosis *I* serogroup and the original antigen. Anti-CFA1-ab reacted with 2 proteins of mw 21 kDa and 45 kDa in the original antigen and a number of proteins with different mw of V. cholerae and Y. pseudotuberculosis. Thus, they were the proteins of mw 67–75 kDa, 54-60 kDa, 43-48 kDa and 29 kDa in V. cholerae Inaba, 67-75 kDa, 60 kDa, 52 kDa, 43 kDa, 29 kDa in V. cholerae Ogawa. Proteins of mw 58 kDa, 50 kDa and 43 kDa were visualised in both V. cholerae 0139 strains while proteins of 37 kDa and 29 kDa were seen only in V. cholerae M045. Proteins of 109 kDa, 0 kDa, 52-56 kDa, 44 kDa, 38 kDa and 31 kDa were visualised in Y. pseudotuberculosis I.

After incubation of the nitrocellulose membrane with polyvalent 01 antiserum anti-CFA1-ab reacted with both of the above-mentioned proteins in the original antigen and proteins of mw 58 kDa, 43 kDa in both V. cholerae 0139 strains. Our data suggest the serological relationships of CFA1 and some proteins with the same (adhesive) activity of enteropathogenic bacteria really exists. This makes E. coli CFA1 a potential candidate for development of a polyvalent safe chemical vaccine against enteric infections and diarrhoeal diseases.

NEW RAPID TESTS FOR DIAGNOSTICS OF UROGENITAL TRICHOMONIASIS AND GONORRHEA

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Dot-ELISA for diagnostics of urogenital trichomoniasis (UT) or gonorrhea (GN) were developed. To obtain specific antibodies an original method of immunisation of BALB/c mice was used. 358 specimens of urethral and vaginal charge from patients with clinical symptoms of urogenital infections were tested in dot-ELISA and a complex of bacteriological methods (direct bacterioscopy and cultivating in special growth media) (BM).

Dot-ELISA gave 1.7 times more positive reactions than BM for UT (287 and 167 cases, respectively) and GN (225 and 128 cases, respectively). When examining patients with mixed (UT+GN) infection (MI) dot-ELISA appeared to be 3.27 times more effective than BM (183 and 56 positive cases, respectively). 27% (97 cases) of GN, 33% (120 cases) of UT and 35.5% (127 cases) of MI were diagnosed only due to dot-ELISA.

The developed test was 2.76 times more effective in the cases of atypical GN (14 positive specimens) in comparison with BM (5 positive specimens) and 26.6 times – in the cases of atypical MI (81 and 3 positive specimens in dot-ELISA and BM, respectively).

No cross reactions with other sexually transmitted diseases were observed (75 specimens).

Duration of analysis was not more than 1.5 hour.

CHARACTERISATION OF *YERSINIA PSEUDOTUBERCULOSIS* STRAINS ISOLATED IN SARATOV REGION, RUSSIA

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In 1999 six *Y. pseudotuberculosis* strains with typical cultural, morphological and biochemical properties were isolated from 3-4 month old calves and piglets with diarrhea in Saratov region, Russia. All of them were lysed with specific *Y. pseudotuberculosis* phage and were agglutinated with commercial antiserum to *Y. pseudotuberculosis III* serotype.

However, when tested in ELISA with a panel of *Y. pseudotuberculosis* type-specific monoclonal antibodies (MCA) obtained previously (Feodorova et al, 1999), only one of them (III72) was stereotyped as *Y. pseudotuberculosis III. Y. pseudotuberculisis III3* reacted with MCA against *Y. pseudotuberculosis VI* serotype. The remaining rest (III5, III71, III191 and III192) appeared to belong to *Y. pseudotuberculosis I* serotype. In all cases MCAs reacted with specific protein epitopes as described previously (Feodorova et al, 1999). The presence of the relevant specific proteins in each strain was shown by the results of 12.5% PAGE-SDS according to Laemmli (1970). Interestingly, the LPS profiles of the examined strains differed from those of the referent strains of Mollaret (1977). All the strains possessed LPS profiles similar to S- or SR- forms of gram-negative LPS, eg, in addition to large zones in the lower part of the gel a number of bands in the middle and upper parts of the gel were visualised.

ELISA test based on the use of these MCAs seems to be valuable for detection and stereotyping of *Y. pseudotuberculosis* in animals.

GENOME POLYMORPHISM OF STRAINS OF *YERSINIA PESTIS* SUBSP. *PESTIS*

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There are 43 natural foci of the plague infection allocated in the territory of FSU (Former Soviet Union). *Y. pestis* strains circulating in the Central Caucasian plague focus, Pre-Caspian Flat and Flat-Foot-Hill plague foci, Central Asian plague foci, Tyan-Shan and Alay plague foci, Transbaikalian and Tuva (Mountain Altai) plague foci, belong to *Y. pestis* subsp. p*estis* according to the classification accepted in Russia. Isolation of phenotypically atypical *Y. pestis* strains from the above mentioned natural foci is the reason for searching new criteria for identification of *Y. pestis* strains from different natural plague foci.

The purpose of this study was to assess the genome polymorphism of 45 strains of *Y. pestis* subs. p*estis* isolated from various natural plague foci of FSU. The 180-bp *Y. pestis* chromosome DNA fragment, which we have cloned previously (Gorshkov *et al*, 1999), served as a genetic probe for Southern hybridisation with *Eco*RI-digested genomic DNA. Higher-molecular-weight bands that were poorly separated were not evaluated, leaving 17-31 distinct bands per strain.

Fingerprinting of the strains currently determined five genetic variants within *Y. pestis* subsp. *pestis*:

(i) *Y. pestis* strains from the Pre-Caspian Flat and Flat-Foot-Hill plague foci.
(ii) *Y. pestis* strains from the Central Caucasian plague focus.
(iii) *Y. pestis* strains from the Central Asia.
(iv) *Y. pestis* strains from the Tyan-Shan and Alay plague foci.
(v) *Y. pestis* strains from the Tuva and Transbaikalian plague foci.

Results of these investigations will allow to perform the molecular-epidemiologic monitoring of *Y. pestis* strains from different natural plague foci.

Gorshkov, O V *et al* (1999) *Mol Genet Mikrobiol Virusol* (Rus) **4**, 29-33.

NOVEL *IN VITRO* DETECTION SYSTEMS FOR THE ANTHRAX TOXIN COMPONENTS

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The characterisation and evaluation of the licensed human anthrax vaccine depend on a variety of *in vivo* tests that determine immunogenicity and protective efficacy of the vaccine. A programme of alternative assessment strategies, based on novel *in vitro* technologies, has been developed. These technologies may be applied to the continued production of a consistent and effective vaccine.

The major vaccine constituents are considered to include the individual toxin components: protective antigen (PA), lethal factor (LF) and oedema factor (EF) and their binary toxins: lethal toxin (LT) and oedema toxin (ET).

Each or all such components may contribute individually or collectively to the potency, toxicity or reactogenicity of the vaccine. This poster will describe the development and application of sensitive, quantitative functional assays that will be capable of discriminating between the binary anthrax toxins (LT and ET) and their individual protein components LF, EF and PA. These *in vitro* detection systems are based on a combination of cell receptor binding and biological activity assays.

Rapid, sensitive and specific assays for the detection of PA, LF, EF and S-layer proteins (Sap and EA-1) will also be described.

THE *BACILLUS ANTHRACIS* S-LAYER PROTEINS EA1 AND SAP ELICIT AN IMMUNE RESPONSE IN HUMANS AND A/J MICE, AND DO NOT ADVERSELY AFFECT THE MURINE IMMUNE RESPONSE TO PROTECTIVE ANTIGEN (PA)

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The UK human anthrax vaccine consists of the alum-precipitated, cell-free culture supernatant of *Bacillus anthracis* Sterne. In addition to the key immunogen, protective antigen (PA), the vaccine also contains a number of other bacterial and media-derived proteins. These proteins may influence the development of the immune response to PA. Bacterial cell-wall components such as S-layer proteins have been shown to be potent immunomodulators and it is known that *B. anthracis* expresses two such proteins, EA1 and Sap.

Two separate studies were carried out on human and mouse sera to examine host immune responses to PA and S-layer proteins and highlight possible adverse interactions between them.

The first study was carried out on human serum samples from infected individuals and others who had received the current UK licensed anthrax vaccine. ELISA data indicated that EA1 and Sap were immunogenic and present in the vaccine.

A co-administration study whereby female A/J mice were given formulations containing EA1 or Sap and recombinant PA (rPA), with or without alhydrogel, showed that the presence of the S-layer proteins had no effect on the mean PA-specific IgG titre in A/J mice. Furthermore, the level of protection conferred by rPA against challenge with *B. anthracis* STI spores was not affected by the presence of EA1 or Sap.

MOLECULAR MECHANISMS IMPLICATING RENAL PROXIMAL TUBULE CELLS IN STAPHYLOCOCCAL ENTEROTOXIN B-INDUCED SHOCK

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The staphylococcal enterotoxins (SE) are of considerable clinical significance as causative agents of food poisoning and lethal shock. The latter is characterised by acute vasodilatation, which results in severe hypotension. SEB administered to primates has been shown to localise in the renal proximal tubule epithelial cells (RPTEC). The role of the kidney in blood pressure management via the renin-angiotensin system has been well established. Furthermore, RPTEC secrete potent vasoconstrictor peptides, endothelins 1 and 2, and possess a highly specific glycosphingolipid-based receptor for SEB, leading to the hypothesis that these cells are involved in mediating SEB intoxication and that SEB-induced apoptosis of RPTEC contributes to the dysregulation of vascular tone associated with SEB-induced shock.

Differential display and reverse transcription polymerase chain reaction studies of the SEB-treated RPTEC cultures revealed altered expression of a number of genes that regulate the production of endothelin -1 (ET-1). Flourescent staining of the RPTEC actin cytoskeleton showed that SEB induced actin stress fiber assembly, which was consistent both with down regulation of RhoE, an inhibitor of stress fiber formation, and up regulation of CSPV. Terminal deoxynucleotidyl transferase-mediated nick end labelling (TUNEL) assay revealed induction by SEB of apoptosis in RPTEC that was consistent with up regulation of SOD-1 and down regulation of L21, both of which had been reported in the literature to be associated with programmed cell death.

Both actin stress fiber assembly and apoptosis induced by SEB were blocked by the inhibitor of Ras farnesyl transferase and phosphotidylinositol-3 kinase (PI3K), thus suggesting their involvement in the molecular signaling cascade activated in RPTEC upon exposure to the toxin. Immunoprecipitation and Western blot studies confirmed that SEB produced activation of PI3K and its downstream target Akt. These findings suggest a model by which renal function may contribute to SE-induced toxic shock.

ISOLATION AND DETECTION OF A WILD STRAIN OF *BACILLUS ANTHRACIS*

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A wild strain of *Bacillus anthracis* was isolated from a clinical case in order to establish a reference culture. The isolated strain was studied for its phenotypical as well biochemical properties. The tests were also conducted for the possession of fimbrae, flagella with motility, self agglutination and penicillin susceptibility. The subcutaneous injection of 100 spores of the isolated culture in to 16 gram mice resulted in death within 90 hrs. Thus, the findings were considered to be significant for the identification of wild strain of *Bacillus anthracis*.

CONSTRUCTION OF A DEFINED LIVE ATTENUATED VACCINE STRAIN OF *FRANCISELLA TULARENSIS*

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Tularaemia is a debilitating disease caused by the facultative intracellular Gram negative bacterium *Francisella tularensis*. The current vaccine is a live attenuated strain of *F. tularensis*, LVS, which was attenuated by repeated passage on artificial media. Although the vaccine offers good protection against fully virulent strains of *F. tularensis*, the molecular basis of the attenuation is unknown. Therefore we aim to construct a rationally defined attenuated mutant of *F. tularensis* to be licensed as a vaccine for general use. In this study we describe our current strategy and our progress. Several genes from the aromatic amino acids (*aroA,aroB,aroC,aroK*) and purine (*purA,purB,guaA*) biosynthetic pathways were selected and were either amplified by the polymerase chain reaction using *F. tularensis* Schu4 template DNA or isolated using Southern blotting techniques. Each of the genes was inactivated by the deletion of a central region of the gene followed by the insertion of a chloramphenicol resistance cassette at the deletion site. Each inactivated gene was cloned into a pUC18-based plasmid or a suicide vector. Deletion constructs will be initially introduced into the fully virulent type B strain, *F. tularensis* HN63 and subsequently into the virulent type A strain *F. tularensis* Schu4 by a method of cryotransformation.

METHOD FOR PREPARATION OF SAMPLES FOR PCR- AND ELISA-DETECTION OF *BRUCELLA* SPP.

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There are different methods of purification of samples from components that inhibit polymerase chain reaction (PCR) and enzyme-linked analysis (ELISA). We developed for this aim an approach based on electrophoretic separation of bacterial cells (or their DNA) and contaminating substrates. In experiments samples of soil, milk, urine and blood, which were artificially infected with Brucella cells in concentration of 10^2-10^7 cfu/ml were used. The electrophoretical separation of components was performed by the method of electrophoresis in free flow of liquid (EFFL) at U=500 V. The following results were obtained. The levels of ILISA– and PCR-detection of brucella cells were the following (as measured in cfu/ml): 10^5 and 10^2 for pure cultures; 10^6 and 10^5 for untreated milk; 10^5 and 10^4 for EFFL-treated milk. The EFFL-treated decreased the amount of PCR-detected cells of brucella in urine – from 10^4 to 10^3 cfu/ml and in blood – from > 10^5 to 10^3 cfu/ml. Thus, the electrophoresis in free flow of liquid is an effective method of separation of components and preparation of different samples for PCR- and ELISA-detection of microorganisms of the genus *Brucella*.

20TH CENTURY ACHIEVEMENTS IN THE CONTROL OF MICROBIAL DISEASES

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The 20th century witnessed a marked decline in infectious diseases in many countries of the world resulting in a decrease in morbidity and mortality. In some nations an increased life expectancy resulted. For example, in the United States the life expectancy increased by about 30 years during the past century attributable to a variety of factors including, to a large extent, advances in the control of microbial diseases. The eradication of smallpox was a public health triumph in the final quarter of the century and is about to be followed by the eradication of poliomyelitis over the next few years. Other microbial diseases are targeted for eradication during the first quarter of the 21st century.

The pioneering accomplishments of Pasteur and Koch identifying microorganisms as causative agents of serious and death-provoking diseases served as the basis for public health activities over the past century resulting in substantial progress in microbial disease control. These efforts centered on (1) improvements in sanitation and hygiene, (2) the discovery and wide-spread use of antibiotics and antiviral agents, and (3) the development of vaccines and the implementation of universal childhood immunisation programs. Each of these areas was highlighted by major technological advances leading to the development of sophisticated strategies for the surveillance and control of microbial diseases.

DETECTION OF BACILLUS ANTHRACIS BY PCR IN THE SAMPLES FROM PATIENTS AND ENVIRONMENTAL OBJECTS DURING AN OUTBREAK OF ANTHRAX IN THE REPUBLIC OF MORDOVIA IN 1999

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An outbreak of anthrax occurred in the Republic of Mordovia in July 1999. Fifty five horned cattle died and 6 patients fell ill with the cutaneous form of anthrax within a fortnight. To carry out laboratory diagnosis and elucidate the cause of the disease among humans and animals, the PCR tests and culturing techniques were sued.

The bacteriologic methods failed to reveal positive cultures in 92 specimens taken from the patients, dead cattle and the environment.

Eight samples from 4 patients treated with antibiotic drugs for 5 to 6 days were examined using the PCR analyses. All the patients were convalescents when the samples were taken from them, however, in every case positive results were observed. Thus the application of PCR tests to diagnose cutaneous anthrax, including antibiotic – treated cases, was shown to be quite suitable.

In the material from two fallen cows (ear blood) the PCR analysis revealed *B. anthracis* DNA.

In order to find out the cause of the anthrax epizootics, 20 environmental samples (grass, soil, water and hay) from the territory of the presumed source of the infection were studied by the PCR method. Positive results were obtained with two of them (fodder grass from a field).

In an attempt to conform these findings, two samples of grass were taken from each of 9 sectors of this field. *B. anthracis* was shown to be present in all the sectors by the PCR method. It was concluded that the source of *B. anthracis* transmission among the cattle was perennial grass (Galega spp) cut in this field situated near an active cattle grave.

Thus, within a two-days period under the conditions of the anthrax outbreak, the PCR analysis was helpful enough to corroborate the clinical diagnosis for the patients and to detect the cause of the epizootics.

SURFACE GLYCOPROTEIN E OF THE TICK-BORNE ENCEPHALITIS VIRUS INTERACTED WITH HUMAN LAMININ BINDING PROTEIN

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The 37 kDa-precursor of laminin binding protein (LBP) was isolated from a human embryo kidney cell line and cloned. The recombinant LBP expressing in *E. coli* cells was purified by Ni-NTA Sepharose chromatography. The purified recombinant LBP interacts with surface glycoprotein E of the tick-borne encephalitis (TBE) virus in Western blot. The binding constant for the interaction of these proteins was 2.51.9 x $10^7 M^{-1}$. The purified recLBP effectively competed with monoclonal antibody 10H10 against the TBE virus for interaction with glycoprotein E. Scanning flow cytometry using FITC-labelled rabbit antibodies against 37 kDa recLBP demonstrated that the highly TBE-sensitive human embryo kidney cell carries not more than 2-3 x 10^5 molecules of the LBP on cell surface. The highly specific interaction of glycoprotein E and recLBP allowed us to conclude that this protein-protein interaction is a ligand-receptor reciprocity needed for the entry of virions of the TBE virus into sensitive cell.

THE NATURAL-CLIMATIC AND SOME SOCIAL FACTORS OF ANTHRAX DISTRIBUTION IN KAZAKHSTAN

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The study of prevalence of the Anthrax in Kazakhstan has shown, that this disease is wide, but is non-uniformly distributed in its various districts and areas. Now in Kazakhstan there are 14 areas with sharp distinctions of natural, soil-climatic conditions, density of the population, intensity of development of animal industries.

For today in Kazakhstan is registered more 3500 unsuccessful anthrax stations. The quantity of them in Kazakhstan districts are from 563 in the South-Kazakhstan district, up to – 7 Mangistauskaya district. Density unsuccessful on the anthrax stations changes – from 4.0 on 1000 sq km (south-Kazakhstan district), up to 0,08 – 0,02 on 1000 sq km (Atyrauskaya, Mangistauskaya district accordingly) and depends on the various factors, including a relief of ground. The more grate density of the foci (on 1000 sq km) is determinated in South-Kazakhstan (4.0), Semipalatinsk (2,0), Jambil districts (1,9) – in a zone high-altitude poyasnosty (belt) of mountains.

Rather there are a lot of foci on unit of the square – in East Kazakhstan, Nord-Kazakhstan, Taldikurhan, Akmola districts (forest- steppe zone, steppe, black earth), but activity it is much lower. In territories of the Aktyubinsk, Karaganda, Pavlodar, West-Kazakhstan districts located in a zone chestnut soils there is low density of anthrax foci (0,1 on 1000 sq km). In a deserted zone of brown and grey —brown soils density of the anthrax foci makes 0.04 on 1000 sq km (Shushayev, 1993).

Last 10 years only about 400 animals was contaminated with anthrax. We noted direct correlation with density of the population, live-stock of agricultural animals and anthrax morbidity of the human and agricultural animals.

It is observed relationship between economic reforms spent in Kazakhstan and anthrax outbreaks. A quota of the people with exposed dangers of anthrax is increase in connection expansion of a private property, reduction of number of vaccination of home animals.

It is registered 9 cases of human anthrax in 1999 in Kazakhstan (index-on 100000 men – 0,06). In 1998 this parameter made – 0.05, and in 1997 – 0,42). Now anthrax human and animal morbidity tends to decrease.

MONITORING OF A LIVE FREEZE-DRYING PLAGUE VACCINE QUALITY

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Since 1960 the main activity of vaccine laboratory of Kazakh Institute of Research on Plague Control is the manufacturer of live freeze-drying plague EV vaccine. Active nature plague foci are situated on 39% of republic territory. Annually above of 49 thousands persons are vaccinated against a plague in Kazakhstan. Last 50 years cases of human plague is registered sporadic in consequence of environmental sanitation of the natural foci and vaccination of the population living on this territory.

Master strain for manufacture of vaccine EV is Y. pestis EV-76 line of Research Institute of standardisation and control of Tarasevich (Moscow).

Comparison of quality of the finish vaccine product during storage has shown dependence its viability from culture medium. A concentration of live vaccine's cells which has been cultivated on mediums prepared on meat hydrolysate was equal 35,4%, and through 6 months of storage at temperature 4°C viability has decreased to 30,5%. Viability of the vaccine which has been inoculated on mediums by usage of a casein hydrolysate was decreased from 30,1% to 19,5%, that of a below regulated metric. A vaccine with such metrics is rejected.

Autoclaving of culture mediums quite often influences of vaccine quality. Viability of a vaccine was reduce from 42% to 35% if bacterial agar was sterilised with 122°C during 30 min instead of 120°C – 30 min.

A viability of a live freeze-drying plague vaccine dependents from places of ampoules in camera for drying. Concentration of live vaccine cells which has been dried up on a lower shelf was higher 10%, than dried up on upper shelf because different temperature.

It is known, that the status of a plague microbe population depends from sunny activity (Gubler, Genkin, 1971; Dubiansky, 1995; Serjanov et al, 1997). We conducted retrospective analysis of vaccine life facility and sunny activity (Wolf's number and 2800 MHz Solar flux). Calculation of the Spirmen's range correlation on 1971-1995 was not revealed any correlation. However, using method of sliding correlative window we revealed that on phases fall down, minimum and animation sunny activity (21-st cycle: 1973-1977, 22nd cycle: 1983-1988) was existed significance correlation (from 0,8 to 1) between indexes life facility of vaccine and Wolf numbers. In this period number of life microbe cells in vaccine 60% was arrived. In period of maximum sunny activity (1978-1982 and 1989-1992) straight correlation changed from –0,8 to –1. In this period number of life vaccine microbe cells 39% was arrived.

Analyses of laboratory data trends is important for a quality of vaccine, that influence on the success of preventative measures.

WAYS OF ISOLATION OF *BACILLUS ANTHRACIS* ASPOROGENIC DERIVATIVES

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We reported previously about selection of the Spo^- derivative from the population of wild strain *B. anthracis* 81/1 (Mikshis *et al*, 1999). The asporogenic clones were stabile and preserved all phenotypic characteristics while cultivated on plating medium and after passaging in laboratory animals.

We found that sporulation-deficient clones of wild strains and some vaccine ones appeared after frequent passaging on nutrient medium. These derivatives were isolated in generations of dissociating clones, which expressed proteolytic, haemolytic activity and adsorption of Congo Red (CR). We used these and others peculiarities of growth on solid and liquid media for selection asporogenic derivatives of vaccine strains *B. anthracis* 55 and Sterne34F2. P Worsham and M Sowers (1999) isolated the asporogenic derivative (SpoOA) of *B. anthracis* ΔSterne-1 (pPA102) using Congo Red agar too.

The protective antigen-producing strain *B. anthracis* STI1 is represented by cells lacking expression of proteolytic, haemolytic activity and adsorption of Congo Red ($Prt^-Hly^-CR^-$) and consequently lacking possibility to form asporogenic clones. Therefore insertion (Tn*917*) Spo^- mutants of nonplasmid derivative of *B. anthracis* STI1 were obtained. High frequency of formation of CR^+ and Spo^- clones was observed after integration of Tn*917* in *B. anthracis* chromosome. The obtained asporogenic mutants of *B. anthracis* STI1ΔT are characterised by absence of the proteolytic activity, that is optimal for the construction of the strains producing the protective antigen.

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SEARCH OF *BACILLUS ANTHRACIS* CHROMOSOME-DETERMINED VIRULENCE GENES

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The virulence of *B. anthracis*, the etiological agent of anthrax, is determined by genes located in residential plasmids and in chromosome. Unlike the plasmid ones, chromosomal determinants of virulence have not been identified yet. Probably, the ability of wild strains of *B. anthracis* to form phenotypes with different expression of degradation of proteinaceous substrates (Prt), haemolytic activity (Hly), adsorption of Congo Red (CR), production of a yellow diffusable pigment (Ydp) and sporulation (Spo) was one of the reasons why chromosomal determinants of virulence could not be identified.

We reported previously about possibility of selection of the stabile phenotypes ($Prt^+Hly^+CR^+Ydp^+Spo^-$, $Prt^-Hly^-CR^-Ydp^-Spo^+$) in populations of different *B. anthracis* strains. Determining their virulence in experiments showed that co-ordinate changes of proteolytic, haemolytic activity, adsorption of Congo Red and production of pigment did not influence essentially on virulence of *B. anthracis*. At the same time, the $pX01^-pX02^+$ derivative of asporogenic clone was at least 100-fold less virulent for white mice than the control ($Prt^+Hly^+CR^+Ydp^+Spo^+$) clone in vegetative form.

The experiments on genetical modelling of virulence of *B. anthracis* were carried out. On the one hand insertion Spo^- mutants (Tn*917*) of nonplasmid derivative of $Prt^+Hly^+CR^+Ydp^+Spo^+$ clone were obtained. On the other hand restoration of sporulation of nonplasmid derivative low virulent Spo^- clone was performed. We used transduction transfer of gene material in recipient cells from two different donors: $Prt^+Hly^+CR^+Ydp^+Spo^+$ and $Prt^-Hly^-CR^-Ydp^-Spo^+$ transductants were received. Study of virulence of insertion Spo^- mutants and constructed Spo^+ transductants on laboratory animals will make it possible to identify chromosomal determinants of virulence of *B. anthracis*.

THE SIGNIFICANCE OF ANTHRAX

A D G Roberts, G Lloyd (CAMR, Salisbury)

Anthrax is primarily a disease of herbivores. Humans acquire it because of contact with infected animals, animal products, or from materials infected with *Bacillus anthracis* spores. *Bacillus anthracis*, the causative agent of Anthrax, is a spore-forming, Gram positive, rod-shaped bacterium approximately 4μm by 1μm. It can survive for long periods, in its spore form, and is commonly referred to as an obligate pathogen. It is enzootic in many countries of Africa and Asia and it occurs sporadically in many other countries including Canada and Australia.

Anthrax infections are still considered important infections world-wide by both physicians and veterinarians. The pressure to develop "brown-field" sites has also raised the profile of environmental investigations required by current legislation. This presentation will cover the current CAMR Anthrax reference service activities and outline the developments made with biological and molecular detection strategies.

Although Anthrax is rare in the UK, the risk of infection from a variety of sources must not be underestimated.

DIAGNOSTIC USE OF PCR FOR DETECTION OF *PNEUMOCYSTIS CARINII* AND *MYCOBACTERIUM TUBERCULOSIS* IN CASES OF PULMONARY INFECTIONS

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Abstract

The world-wide increase in tuberculosis and pneumonia and the emergence of multidrug-resistant pathogens have demonstrated the weaken in the currently used techniques and underscored the need for more rapid and accurate methods of laboratory diagnosis. In this study, the nested PCR protocol for detection of both *Pneumocystis carinii* and *Mycobacterium tuberculosis* DNA in clinical specimens [broncoalveolar lavage (BL), sputum and pleural fluid] was evaluated. A total of 150 BAL, 148 sputum and 7 pleural fluid specimens from 298 patients suspected of having pulmonary infections (Bronchitis, TB, pneumonia and others) were preliminary diagnosed for presence of *M. tuberculosis* and *P. carinii* by both Zeil-Neelsen and Giemsa stain respectively. Further molecular diagnosis of both infectious organisms were also evaluated by PCR. Results revealed that only 25 specimens out of 305 specimens were positive for *M. tuberculosis* by Zeil-Neelsen stain and PCR assay. However an additional of 6 specimens were positively diagnosed for *M. tuberculosis* by PCR although they were preliminary negatively diagnosed by smear staining. *P. carinii* was not seen under microscopic examination, nor is expected by using the PCR assay, due to the rare cases of immunocompromised patients mostly AIDS, in Jordan. PCR assay actually will reduce the time needed for specimen analysis compared to conventional methods and could be applied for early and specific diagnosis.

STUDY OF DISINFECTANT PROPERTIES OF *ASPERGILLUS SP.* CULTURE EXTRACTED FROM COLORADO BEETLE

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Microbiological industry requires regular input of highly active strains, the main source of which still remains natural isolates.

Form larva of Colorado beetle, one of the dangerous pest of potatoes, the culture *Aspergillus sp.* which has antagonistic properties related to phytopathogenic micro-organisms, such as *Fusaium oxysporum, F. solani. Rhizoctonia solani, Sclerotium sclerotiorum, Alternaria solani, Erwinia carotovorum* was isolated.

Further, in the study were examined disinfectant properties of isolate obtained. As the test-organisms eggs of Colorado beetle as well as larvae of 1-4 age were taken.

Testing was conducted in laboratory conditions in vitro. The laboratory tests have shown, that the processing of egg-laying, during 5 mines by aqueous suspension of *aspergillus sp.* culture, precludes birth of larvae. Under a binocular was observed tightening of eggs and their pasting. Spraying of larvae of 1-2 age and their fed by cultural liquid of isolate has led to death from 12 to 20% of larvae in 3 days. Injection of suspension culture in oral cavity of larvae of 3-4 age has reduced to stopping of movement of 70% and death of 30% of larvae in 72 hours. In control variants (water) under the same conditions of experience death of larvae was not detected.

The researches mentioned above appeared to be the for further studies of new isolate with the purpose of detection of a complex of biologically active substances and sampling of compounds, in charge of for antiparasitic activity. So, the chromatographic analysis has revealed series of phenolic compounds, such as flavones naphthols, flavonoids, oxycompound, amino acids, polysaccharides, polypeptides etc. Some of the compounds were extracted in the pure state and are tested as possible insecticides.

RECOMBINANT EXPRESSION OF ANTHRAX COMPONENTS

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Anthrax continues to be a problem both in the case of naturally and occupationally acquired infections by *Bacillus anthracis*. Oedema Factor [EF], Lethal Factor [LF] & Protective Antigen [PA] are the three anthrax toxin antigens which are involved in both infections and are also the main constituents of the anthrax vaccine.

A series of new expression vectors for production of the three anthrax toxin components were constructed. The new expression vectors used one of two fusion proteins at the N-terminus to aid purification. One system used the synthetic IgG binding domain (ZZ-domain), whilst the other used the first 20kDa of the protective antigen (PA) protein and a hexa-histidine tract for purification. The non-toxigenic host (*B. anthracis* UM23C1-1) was employed for expression. The advantages of using a native host for expression of these proteins were that high levels of expression were achievable without the problems of proteolytic cleavage observed with alternative expression hosts. This novel system has allowed the production of the separate antigens, which are highly purified and antigenically reactive.

Expression using both systems was similar in terms of total recovery of recombinant protein. Purification using the ZZ-domain fusion proteins rather than using the hexa-histidine tract was found to give more rapid, easier purification of toxin proteins.

These non-infectious antigens have proved valuable tests in the development of laboratory diagnostic strategies and the characterisation of biological and molecular vaccines.

EXTRACELLULAR PROTEASES OF *BACILLUS ANTHRACIS*

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Fully virulent strains of *Bacillus anthracis*, the causative of anthrax possess two, well characterised plasmid encoded virulence factors. A tripartite toxin complex encoded by pX01 and a poly D-glutamic acid capsule located on pX02 (Mikesell *et al*, 1983). Attenuated strains can be created by the deletion of either pX01/2.

However a basal level of virulence is present, as demonstrated by infecting mice with plasmid cured *B. anthracis* and *B. subtilis*. While the *B. subtilis* strains were destroyed rapidly, the *B. anthracis* strains persisted in the hosts (Pezard *et al*, 1991). This work suggested that other virulence determining genes may be located in the chromosome of *B. anthracis*.

A number of strains of *B. anthracis* have been isolated which lack proteolytic activity, these also possess reduced virulence in comparison to the wild-type strains. It is widely recognised that extracellular metalloprotease production is a key virulence determinant in a variety of pathogenic bacterial species (Hase & Finkelstein, 993). The difference in virulence levels amongst *B. anthracis* strains may therefore be due to the activity of extracellular metalloproteases.

Known extracellular protease genes from *B. subtilis* (http://genolist.pasteur.fr/SubtiList) were used to identify putative extracellular protease genes within the *B. anthracis* genome (http://www.tigr.org).

A high degree of sequence homology was identified in the neutral protease (*nprE*) gene of both species. The contribution of nprE to the total extracellular protease levels was analysed using resorufin labeled casein assays in the presence of a variety of protease inhibitors. At the genomic level the *nprE* gene was amplified, restriction mapped and sequenced from a variety of *B. anthracis* strains.

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DNA VACCINATION AS PROPHYLAXIS AGAINST ANTHRAX

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Biolistic introduction (ie by "gene gun") of "naked" DNA encoding a foreign protein antigen into the skin of mice was shown in 1992 to result in the induction of an antibody response, due to the antigen being expressed in an immunogenic form (Tang *et al*, 1992). Subsequent research demonstrated that intramuscular injections of plasmid DNA encoding influenza nucleoprotein protected mice against challenge with live influenza virus (Ulmer *et al*, 1993).

The aim of this series of experiments was to determine if immunisation with DNA encoding protective antigen (PA), followed by a single booster dose of PA protein could protect A/J mice against a subsequent challenge with *Bacillus anthracis*. Mice were vaccinated either with the PA gene from *B. anthracis*, or a PA gene resynthesised with an *E. coli* codon bias.

Immunisation with a single dose of PA with Freunds incomplete adjuvant resulted in an anti-PA IgG subclass profile that was predominantly IgG1, indicative of a Th2-type response. A similar profile was seen in the DNA vaccinated mice, both before and after dosing with PA protein.

Three doses of either DNA construct induced an anti-PA titre of at least 2 logs higher than that seen following a single dose of PA protein. Subsequent dosing with PA protein induced a further increase in anti-PA IgG subclass titres, with the smallest percentage increases observed in the IgG1 subclass.

Following challenge with the STI strain of *B. anthracis* (10^5 or 10^6 spores delivered ip), all vaccinated animals survived whilst all the negative control animals died.

This study concluded that gene gun delivery of DNA constructs dramatically increased the antibody response compared to intramuscular delivery of PA protein alone and was able to give complete protection against *B. anthracis*

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CLONING AND EXPRESSION THE pncB GENE FROM MYCOBACTERIUM AVIUM

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A test commonly used to distinguish human strain of M. tuberculosis from bovine and non-tuberculosis mycobacteria (NTM) is the nicotinic acid (NA) test. Human varieties of M. tuberculosis accumulate large quantities of NA in the culture medium, whereas other mycobacteria do not. A study revealed that extracts prepared from a human strain of M. tuberculosis had very high level of nicotinamide adenine dinucleotide (NAD) glycohydrolase and nicotinamide deamidase when compared with the bovine strain. Furthermore, whereas enzyme preparations exhibited activity with regard to enzyme of biosythetic pathway from quinolinic acid (QA) through nicotinamide adenine dinucleotide (NAD), nicotinic acid phosphoribosyltransferase (NAPRTase) levels were extremely low or absent. The human strain of M. tuberculosis rapidly degrades NAD to NA but cannot recycle the NA to NAD. The NA then accumulates extracellularly. The bovine strain, also with low NAPRTase activity, dose not degrade NAD as rapidly, thereby accumulating much less NA.

In order to understand the molecular mechanism of nicotinic acid phophoribosyltransferase (NAPRTase), we screened the Jigt11 genomic library of M. avium and cloned, expressed the pncB gene encoding for this enzyme.

We designed a pair of primers according to known M. tuberculosis pncB gene and amplified 1.8kb fragment with PCR by using M. tuberculosis genomic DNA as template. Label the fragment using the E-32P random primers as probe for screening the M. avium Jigt11 genomic library.

Plate the diluted library to top agarose agar of LB medium and place the inverted plates at 37°C overnight to allow the plaques to form. Transfer the plaques onto nylon membrane and hybridised with labeled probe. Compare the results between autoradiography and phage plaque growing LB plates, we got the positively hybridised plaques. After amplifying growth, extract the phage DNA; made the restriction map and do Southern blot again. We obtained one 3.5kb and 2.5kb positively hybridised DNA fragment respectively. Subcloning these two DNA fragment into the plasmid pUC19 and sequencing, we got the whole pncB gene sequence of M. avium. It is 1491 bp long and deduced protein contain 497 amino acid. Its molecular weight is 54.3kd. After homologous search, its homologous rate with M. tuberulosis pncB protein is 67%. Ligated the M. avium pncB gene to the expression vector pTrcHis and transformed into E. coli. The expressed product was sonicated and subject to SDS-PAGE, we got the expressed protein with expected size. This investigation will facilitate the study of NAD metabolism of mycobacteria.